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Drosophila ananassae inhabits most of the tropical and subtropical regions of the world. In contrast to *D. melanogaster* and *D. simulans*, populations of *D. ananassae* exhibit a distinct genetic population substructure through most of their geographic range. Studies of *D. ananassae* populations from Trinity Beach (Australia), Apia (Samoa), Nadi (Fiji) and Java (Indonesia) and its sister species, *D. pallidosa* from Malololelei (Samoa) and Nadi (Fiji) showed significant levels of prezygotic mating discrimination. However, it is unclear whether postzygotic isolation exists, and if fitness of hybrids from matings between populations and between *D. ananassae* and *D. pallidosa* is lower than fitness of offspring from matings within populations. Such postzygotic reproductive isolation among populations of *D. ananassae* would indicate that the populations may be in the early stages of speciation. In this study, I determined the extent to which postzygotic reproductive barriers exist among populations of *D. ananassae* and *D. pallidosa* from Australia, Samoa, Fiji, and Indonesia. I measured hybrid sterility and hybrid inviability as components of hybrid fitness of offspring from crosses between populations of *D. ananassae* and *D. pallidosa*. I found there is measurable postzygotic isolation between *D. ananassae* and *D. pallidosa* as species, but that there little measurable postzygotic isolation among populations of *D. ananassae*.

POSTZYGOTIC SEXUAL ISOLATION AMONG POPULATIONS OF *DROSOPHILA ANANASSAE* AND *DROSOPHILA PALLIDOSA* FROM INDONESIA, AUSTRALIA, FIJI, AND SAMOA

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CHAPTER I

INTRODUCTION

Dobzhanzky (1940) first proposed that if two populations share a common geographic range, then mutations will appear in either that cause the mutants to be less likely to mate with the opposite population. This leads to two immediate implications; First, if the mutant gene causes a behavioral or physiological change that only allows the carriers to mate within their own population, the gene may spread steadily throughout the population by drift until it becomes fixed. Conversely, if the mutant gene allows the carrier to mate with the opposite population, but there is any hybrid disadvantage, the lowering of fitness in offspring from parents of differing populations, the mutant gene may spread through the population by natural selection. That gene or combination of genes causes reproductive isolation between closely related species.

Models of Speciation

In recent years, the idea of species and speciation has taken on tremendous importance. Throughout the world, companies and scientists are patenting entire species for their commercial value. In this atmosphere, it is essential to determine precisely what defines a unique species. Though there are no universally accepted definitions of a species, there are three models that are generally agreed to be modes of speciation: allopatric, parapatric, and sympatric.

The allopatric model of speciation, first proposed by Mayr (1942), involves the physical separation of a population into two or more groups by a geographic barrier. The lack of gene flow and gradual genetic changes that occur by mutation, genetic drift, and natural selection cause changes that eventually define each population as a new species (Mayr 1982; Mayr and Ashlock 1991).

In the parapatric model of speciation, new species evolve from a single, geographically large and contiguous population. This model involves the establishment of a hybrid zone between the extreme edges of the population. From the extreme edges of the population, there may be a stepped cline in allele frequency or morphological characteristic. The morphology and genetic characteristics of the population change along a gradient from one extreme to another until the forms at either end can be classified as separate species (Harrison 1993). A classic example of parapatric speciation is the hooded crow (*Corvus corone*) and the carrion crow (*Corvus cornix*). The hooded crow is common in Western Europe while the carrion crow is common in Eastern Europe. In the center of Europe, the range of the two species overlaps to form a hybrid zone. On either side of the hybrid zone, the crows can be easily distinguished as separate species, but in the hybrid zone that distinction cannot be made (Ridley 1996).

Sympatric speciation involves the evolution of two species without the home range of the parent population being separated by a physical barrier. Though this model is controversial, there is evidence that it may have occurred in *Drosophila silvarentis* and *Drosophila heedi*. Both species of *Drosophila* live on the same type of tree, but they never interact as one lives close to the soil and the other higher on the trunk (Kaneshiro

1976). It was shown that *Drosophila simulans*, *Drosophila melanogaster*, and *Drosophila immigrans* all utilize the same fruit for food and oviposition at different times in the fruit's decomposition (Parsons 1981). These closely related species of *Drosophila* have differing abilities to process the ethanol produced by the fruit as it decomposes. When the fruit becomes inhospitable to one species, another will take its place. This has led to competition within the species for the resource, but not to competition between species. A third experiment demonstrated that disruptive selection within a species living in sympatry causes reproductive isolation. Rice (1984) found that, when allowed to choose their own habitat within the lab, populations of *D. melanogaster* that occupied a specific habitat displayed temporary prezygotic isolation that, after 29 generations, evolved into true postzygotic isolation. That is, over time, *Drosophila* that chose to live high in an enclosure became reproductively isolated from *Drosophila* that chose to live near the floor of the enclosure. These experiments all show that even when there is no physical barrier to reproduction, reproductive isolation may evolve between populations with overlapping ranges.

Prezygotic and Postzygotic Reproductive Isolating Mechanisms:

Though the models of speciation are well-defined, there are two distinct methods of reproductive isolation occurring either simultaneously or individually that drive the models. The first method, prezygotic isolation, generally involves a change in pre-mating signals and most often occurs prior to any other isolation. Postzygotic isolation occurs after mating has concluded and usually involves a reduction in the fitness of the

resultant offspring. Either of these mechanisms will lead to increased reproductive isolation and divergence between the populations until two unique species occupy the niche previously filled by one.

A population's adaptation to local geographic regions can correspond to an increase in reproductive isolation if gene flow with other populations is restricted, allowing genetic differences to accumulate (Halliburton 2004). Once gene flow is no longer restricted, the populations may not be able to interbreed, in which case they have become different species. Alternatively, hybrid offspring from matings between the populations previously in allopatry may have lower fitness than the offspring from matings within each population. In such a case, reproductive isolation will proceed at an accelerated pace as the mating isolation is reinforced (Dobzhanzky 1940).

Prezygotic reproductive isolation occurs prior to the insemination of the female and may be caused by an interruption in mating rituals, a change in pheromone, or an alteration of any other mating signal a species may use (Schluter 2001; Noor 1999; Futch 1966). Prezygotic isolating mechanisms have been well documented in *D. mauritiana* and *D. simulans,* (Coyne 1989), *D. melanogaster* (Wu et al. 1995; Hollocher et al. 1997), and *D. persimilis* and *D. pseudoobscura* (Noor 2004). Prezygotic barriers serve to reinforce species boundaries and may be a necessary step toward a population dividing into species.

Postzygotic reproductive barriers occur after fertilization between two species. If the hybrid zygotes of the cross fail to develop (hybrid inviability), there will be no viable offspring in the next generation. If the hybrid offspring do mature but are incapable of

reproduction (hybrid sterility), they will be unable to contribute their genes to the next generation.

The two most commonly observed phenomena noted concerning fitness effects in hybrid offspring that drive speciation are Haldane's Rule and the disproportionate effect of the X chromosome. Haldane's Rule stipulates that whenever two different populations mate and one sex is missing, rare, or sterile, that sex is the heterogametic sex (Haldane 1922). Haldane's Rule is a common feature of many species (Gray 1954; Gray 1958, Bock 1984). Of 145 *Drosophila* species examined, 141 followed Haldane's Rule (Orr 1988). In *Drosophila pseudoobscura* and *Drosophila persimilis,* hybrids produced fertile females and sterile males (Orr 1987). Backcrosses produce fertile females and occasionally sterile males. Through chromosome mapping, Orr determined that the sex chromosomes were the main cause of the sterility in males (Orr 1987; Orr 1989), consistent with the predictions of Haldane's Rule.

Both Haldane's Rule and the Large X Effect were further demonstrated by a process similar to Orr's by Coyne (1989). Coyne found that males always become sterile or inviable in all of the crosses before any females. Though hybrid sterility usually only occurs in the heterogametic sex of the offspring, a simple reduction in the fitness of the hybrid, not as extreme as hybrid sterility or hybrid inviability, could result in the long-term development of isolation. This reduction in fitness may be caused by a disruption of gene combinations (coadapted gene complexes) or a high accumulation of deleterious gene combinations. Any of these mechanisms may be responsible for the postzygotic mechanism of speciation between two populations.

There is evidence that prezygotic reproductive isolation occurs prior to postzygotic reproductive isolation. Coyne and Orr (1997) examined patterns of speciation in *Drosophila*. They make several conclusions based on a large data set consisting of the Nei's genetic distance estimated from gel electrophoresis, prezygotic isolation indexes, and postzygotic isolation indexes of 69 different species crosses. Their results indicate that prezygotic and postzygotic isolation increase with time. They also find that prezygotic isolation is stronger than postzygotic isolation only in sympatric taxa. Further, they demonstrate that there is a large lag time between the appearance of hybrid sterility/inviability in males than in female hybrids. These findings were supported by Boake (2000) who found that *D. silvestris* and *D. heteroneura,* two sympatric species, display a significant amount of prezygotic isolation even though studies of hybrids between the two species suggest no evidence of postzygotic reproductive isolation (Boake 2000). All of these studies suggest that *Drosophila* species are continually diverging to produce reproductively isolated populations by means of prezygotic isolation, eventually leading to speciation expressed as postzygotic reproductive isolation.

D. ananassae, a Model Organism for Speciation Studies

Drosophila ananassae is an ideal model organism to examine the mechanisms of early speciation. *D. ananassae* is a cosmopolitan species, covering a large geographic area throughout most of the tropical and subtropical regions of the world. Ancestral populations are believed to have originated in Southeast Asia, and have subsequently

colonized many geographic regions of the world (Dobzhansky and Dreyfus 1943, Vogl 2003, Das 2004). Populations of *D. ananassae* are semi-isolated, and studies of DNA sequence polymorphism suggest that positive Darwinian selection has had a significant impact on the genome of *D. ananassae* populations from Sri Lanka, Nepal, Myanmar, and India (Chen 2000; Stephan 1998). These studies indicate that each local population of *D. ananassae* may have adapted to its unique geographic region.

Genetic studies of *D. ananassae* populations, including isozyme polymorphisms (Johnson 1966; Johnson 1971; Tobari 1993), chromosomal inversions (Futch 1966; Tobari 1993), and DNA polymorphisms (Stephan and Langley 1989; Vogl 2003, Das 2004; Baines 2004, Schug et al. 2007, 2008), indicate that populations across large geographic regions are highly structured. By examining the genetic structure of a population, it was determined that small, isolated units have higher evolutionary potential (Patton 1989). In populations with a distinct genetic structure, speciation may occur more quickly due to new gene combinations that do not occur in the ancestral species range that may then become fixed in the population by genetic drift. If there is gene flow between the populations, evolutionary divergence is constrained (Slatkin 1987). The genetic differentiation implied by the structured populations has led some to speculate that subpopulations *of D. ananassae* are, in fact, geographic races (Tobari 1993) with a genetic cline between the disparate populations (Stephan 1998). This indicates that each subpopulation of *D. ananassae* may have adapted to its unique local geographic region, leading to the initial stages of speciation.

Stephan and Langley (1989) assayed two loci for three populations of *D. ananassae* and recognized that they have limited gene flow between them. This led to another series of works in which 4 populations of *D. ananassae* had 3 gene sequences analyzed. Two of the populations existed in a subtropical climate while the other two lived in a temperate climate (Stephan 1998; Chen 2000). Though there was little differentiation between the populations for the *Om (1D)* gene, the frequency of *furrowed (fw)* and *vermillion (v)* were very different between the subtropical populations and the temperate populations, but were consistent within their climate. This could indicate that there may be an adaptive mutation to the climate around the *fw* and *v* loci, or it could merely show that the populations are structured by their climate. These experiments also show that, despite the limited gene flow between populations, there is still genetic differentiation between *D. ananassae* populations and this differentiation due to local conditions could result in reproductive isolation

It has been demonstrated that ancestral populations of *D. ananassae* are from Indonesia (Vogle et al. 2003). During the past 20,000 years it colonized Australia and the South Pacific islands possibly accompanying human colonization of these geographic regions. The population structure of the *Drosophila* in this region described by Schug et al. (2007, 2008) based on mtDNA haplotype and nucleotide diversity supports this hypothesis. There are distinct genetically related clusters within the species range that correspond to physcially isolated geographic regions. *D. pallidosa*, a sister species of *D. ananassae* first described by Bock and Wheeler (1972), has been identified only in Samoa, Tonga, and Fiji, and is differentiated from *D. ananassae* by a lighter body color

in the South Pacific geographic region and fewer bristles on the sex combs of males. The *D. ananassae* from Indonesia appear centrally in most cladograms while those from Australia cluster together and those from the South Pacific cluster together. The Apia, Samoa population examined in this study appears as a relatively genetically divergent group and it appears that it is more distinct from both the ancestral population in Indonesia and other South Pacific populations than *D. pallidosa* (Schug 2007, 2008).

Stone (1966) analyzed both parental and hybrid fitness in *D. ananassae* populations from Tutuila and Palmyra including viability, fertility, and fecundity. Female fecundity was measured as the number of eggs laid per day over a four to six day period. Female fertility was measured as the number of pairs who produced any offspring. Viability was measured as the percentage of eggs laid that eventually pupated. Stone found that the light and dark form of *D. ananassae* from Apia, Samoa show complete prezygotic reproductive isolation, but will produce fertile offspring in no choice experiments indicating that the major cause of the reproductive isolation between these forms is sexual. Stone found that there appeared to be a reduction in fertility and egg development in the initial cross test, but was unable to quantify its extent due to uncontrolled laboratory conditions.

Recently, Schug et al. (2008) have shown that there is significant premating isolation among populations of *D. ananassae* and *D. pallidosa* inhabiting Australia, and Samoa by performing multiple-choice mating experiments between five populations of *D. ananassae* from Java, Indonesia, Thursday Island, Australia, Trinity Beach, Australia, Apia, Samoa, and Malololelei, Samoa. They concluded that the high levels of premating

isolation among these populations suggest that they may be in the initial stages of speciation. Based on the populations surveyed by Schug et al. (2007, 2008), I hypothesize that there are significant differences in the number of offspring produced by hybrids between these populations and the parental population control crosses, and that there is a difference in the number of offspring produced between backcrosses of hybrids to one parent or the other. I expect that hybrid males will produce fewer offspring than hybrid females due to Haldane's rule and the large X effect.

In this study I tested the hypothesis that hybrids of *D. ananassae* populations from the ancestral and South Pacific geographic range that show patterns of mate discrimination also display reductions in hybrid viability using the number of offspring produced as a proxy for viability. I did not include other measures of viability such as egg production and survival of fertilized eggs to adulthood. Throughout this dissertation I use viability to refer to the number of offspring produced that survived to adulthood. I measured the viability of F1 hybrids between species and between populations and the viability of F2 (offspring of the hybrids), to determine if either sex of the F1 hybrid is sterile.

CHAPTER II

MATERIALS AND METHODS

Samples

The postzygotic isolation among populations from Trinity Beach, Australia and Java, Indonesia, and sympatric populations of *Drosophila ananassae* and *Drosophila pallidosa* from Nadi, Fiji and Apia (*D. ananassae*) and Mallololelei (*D. pallidosa*), Samoa (Table 1) were crossed to test for hybrid inviability. Isofemale lines were established from these populations (Table 1) and were maintained as cultures in a Percival incubator with a consistent moisture (~80%) and temperature of 25°C. All of the flies were reared in 3 x 10.5 cm vials containing yeast/agar/sucrose medium under a 14:10 hr. light/dark cycle. A small number of flies were placed in each vial to avoid crowding. Ampicillin (100 mg/l) was frequently added to the food to control for infections.

Table 1: The location of collection sites for the population samples used in this study.

Sampling Location	Country	Abbreviation	Date Collected	Latitude and Longitude	Collector
Nadi	Fiji	N	2005	17° 47°S, 177° 29° E	M. Schug, S. McEvey, S. Smith, M. Marshall
Nadi	Fiji	P	2005	17° 47°S, 177° 29° E	M. Schug, S. McEvey, S. Smith, M. Marshall
Trinity Beach	Austrailia	T	2003	16° 55' S, 145° 46' E	M. Schug, S. McEvey, S. Smith, M. Marshall
Apia	Western Samoa	A	2003	13° 50' S, 171 °44' W	M. Schug, S. McEvey, S. Smith, M. Marshall
Bogor, Java	Indonesia	B	2001	6° 34' S, 106° 45' E	Aparup Das and Lab
Malololelei	Western Samoa	M	2003	13° 54' S, 171° 45' W	M. Schug, S. McEvey, S. Smith, M. Marshall

Figure 1: Geographic Map of the Collection Sites

No-choice mating experiments were performed to measure differences in the viability of both homospecific and hybrid crosses as measured by the number of offspring produced per cross. To eliminate any possible line effect regardless of collection location, six randomly chosen isofemale lines from each species were tested in each experiment. To remove the effect of inbreeding depression, the males and females to be mated were not taken from the same isofemale line whenever possible.

Ten days after the cultures were set up, 10 males and 10 virgin females were collected from each chosen isofemale line and maintained on fly media for 3-5 days prior to crossing with another line to ensure that females were virgins. This period of time allowed all of the flies to sexually mature (Tobari, 1993). The postzygotic isolation experiments started in the late afternoon, after the dark cycle had begun in the incubator. Approximately thirty males and thirty virgin females, ten flies per isofemale line, were released into a 6 oz. square bottom polypropylene bottle filled with approximately 25 ml. of fly media. The flies were then placed in the incubator overnight. The next day, in the early afternoon approximately 4 hrs after the beginning of the light cycle, all of the flies

were siphoned into individual vials and returned to the incubator. After six days, the females with larvae present in their vials were discarded and the vials placed back in the incubator. Every day thereafter, the vials were checked for newly emerged offspring, quantifying the number of males and females eclosing each day until no new flies were found in any of the vials in that cross.

I tested viability of the F1 offspring using the same techniques as above. The data gathered from the hybrid crosses provided a measure of relative fitness of hybrid offspring when compared to the control group of parental crosses. All of the experimental, control, and reciprocal crosses were completed in the same manner. The number of crosses performed per population is displayed in Table 2 and Table 3.

Further no-choice experiments were designed to test whether hybrid crosses from *D. pallidosa* and *D. ananassae* from Malololelei and Apia, respectively, produced significantly different numbers of offspring compared to the parental crosses. In these experiments, the flies were collected as noted above, but rather than being placed en masse in square bottom polyurethane bottles, 2 males of one population were crossed with 1 virgin female of a different population in smaller, 3 x 10.5 cm vials containing yeast/agar/sucrose medium collection tubes plugged with cotton. The experimental unit for these experiments is the individual female parent. The experiments were performed in this manner to allow for each individual container to count as a separate no-choice experiment.

Table 2: The number of crosses performed per population in the F1 generation.

Male	Female	Number of Crosses
Apia	Apia	17
Apia	Bog	39
Apia	Nadi	20
Apia	Pallidosa	21
Apia	Trinity Beach	13
Bog	Apia	9
Bog	Bog	16
Bog	Nadi	22
Bog	Pallidosa	9
Bog	Trinity Beach	24
Nadi	Apia	9
Nadi	Bog	17
Nadi	Nadi	12
Nadi	Pallidosa	10
Nadi	Trinity Beach	10
Pallidosa	Apia	4
Pallidosa	Bog	14
Pallidosa	Nadi	4
Pallidosa	Pallidosa	19
Pallidosa	Trinity Beach	10
Trinity Beach	Apia	25
Trinity Beach	Bog	18
Trinity Beach	Nadi	14
Trinity Beach	Pallidosa	5
Trinity Beach	Trinity Beach	26

Table 3: The number of crosses performed per population in the F2 generation. For hybrid individuals, the male parent of the hybrid is listed first and the female parent is listed second (ex. Nadi-Bog is a hybrid between a male from the Nadi population and a female from the Bog population)

Male	Female	Number of Crosses
Apia	Apia-Apia	15
Apia	Apia-Bog	24
Apia	Apia-Nadi	11
Apia	Apia-Pallidosa	4
Apia	Apia-Trinity Beach	12
Apia	Bog-Apia	4
Apia	Nadi-Apia	4
Apia	Pallidosa-Apia	3
Apia	Trinity Beach-Apia	10
Apia-Apia	Apia	19
Apia-Bog	Apia	10
Apia-Bog	Bog	12
Apia-Nadi	Apia	13
Apia-Nadi	Nadi	5
Apia-Pallidosa	Apia	4
Apia-Pallidosa	Pallidosa	7
Apia-Trinity Beach	Apia	5
Apia-Trinity Beach	Trinity Beach	4
Bog	Apia-Bog	10
Bog	Bog-Apia	11
Bog	Bog-Bog	68
Bog	Bog-Nadi	15
Bog	Bog-Pallidosa	6
Bog	Bog-Trinity Beach	5
Bog	Nadi-Bog	15
Bog	Pallidosa-Bog	4
Bog	Trinity Beach-Bog	5
Bog-Apia	Apia	4
Bog-Apia	Bog	13
Bog-Bog	Bog	40
Bog-Nadi	Bog	7
Bog-Nadi	Nadi	5
Bog-Pallidosa	Bog	30
Bog-Pallidosa	Pallidosa	7
Bog-Trinity Beach	Bog	8
Bog-Trinity Beach	Trinity Beach	9
Nadi	Apia-Nadi	7
Nadi	Bog-Nadi	3
Nadi	Nadi-Apia	4
Nadi	Nadi-Bog	7
Nadi	Nadi-Nadi	23
Nadi	Nadi-Pallidosa	5
Nadi	Nadi-Trinity Beach	10
Nadi	Pallidosa-Nadi	4
Nadi	Trinity Beach-Nadi	6
Nadi-Apia	Apia	15
Nadi-Apia	Nadi	12
Nadi-Bog	Bog	6
Nadi-Bog	Nadi	4
Nadi-Nadi	Nadi	16
Nadi-Pallidosa	Nadi	4
Nadi-Pallidosa	Pallidosa	4
Nadi-Trinity Beach	Nadi	9
Nadi-Trinity Beach	Trinity Beach	13
Pallidosa	Apia-Pallidosa	3
Pallidosa	Bog-Pallidosa	11
Pallidosa	Nadi-Pallidosa	14
Pallidosa	Pallidosa-Apia	5
Pallidosa	Pallidosa-Bog	3
Pallidosa	Pallidosa-Nadi	5
Pallidosa	Pallidosa-Pallidosa	52
Pallidosa	Pallidosa-Trinity Beach	10
Pallidosa	Trinity Beach-Pallidosa	3
Pallidosa-Apia	Apia	3
Pallidosa-Apia	Pallidosa	4
Pallidosa-Bog	Bog	4
Pallidosa-Bog	Pallidosa	3
Pallidosa-Nadi	Pallidosa	11
Pallidosa-Nadi	Nadi	3
Pallidosa-Pallidosa	Pallidosa	68
Pallidosa-Trinity Beach	Pallidosa	18
Trinity Beach	Apia-Trinity Beach	29
Trinity Beach	Bog-Trinity Beach	3
Trinity Beach	Nadi-Trinity Beach	16
Trinity Beach	Pallidosa-Trinity Beach	5
Trinity Beach	Trinity Beach-Apia	18
Trinity Beach	Trinity Beach-Bog	9
Trinity Beach	Trinity-Beach-Nadi	7
Trinity Beach	Trinity Beach-Pallidosa	3
Trinity Beach	Trinity Beach-Trinity Beach	16
Trinity Beach-Apia	Apia	7
Trinity Beach-Apia	Trinity Beach	11
Trinity Beach-Bog	Bog	22
Trinity Beach-Bog	Trinity Beach	14
Trinity-Beach-Nadi	Nadi	4
Trinity-Beach-Nadi	Trinity Beach	7
Trinity Beach-Pallidosa	Pallidosa	4
Trinity Beach-Pallidosa	Trinity Beach	6
Trinity Beach-Trinity Beach	Trinity Beach	12

Analysis of Postzygotic Isolation

The questions that will be answered by this study are 1) Do hybrid crosses between *D. ananassae* and *D. pallidosa* show a reduced viability, 2) Do hybrids between *D. ananassae* from the South Pacific peripheral range and the Indonesian ancestral range show a reduced viability, 3) Do populations *of D. ananassae* and *D. pallidosa* that live in sympatry display postzygotic isolation, 4) Do the populations of *D. pallidosa* and *D. ananassae* examined display postzygotic isolation based on the Coyne and Orr (1989,1997) isolation index, and 5) Do semi-isolated populations of *Drosophila ananassae* display measurable pre-mating isolation prior to displaying measurable postzygotic reproductive isolation? The data gathered from these crosses consists of the number of emergent adults per hybrid cross (Appendix A.). Because there is such a large variation in the number of offspring produced between each experimental date, I used a linear mixed model with date as a random factor (Appendix B.). A reduced model was created that excluded the hybrid male crosses and hybrid female crosses from the model (Appendix B.) and an ANOVA was performed between the full and reduced models using square root transformed data to meet the assumption of a normal distribution (Appendix C.).

Three models were created to examine the first three questions. The first linear mixed model compared the number of offspring produced by the parental crosses to the number of offspring produced by the hybrid crosses (Appendix B.). In this case, the reduced model provided no distinction in the class for the total number of offspring (Appendix C.) and the ANOVA tested the null hypothesis that the mean number of

offspring produced by the hybrids is not different than the mean number of offspring produced by each species.

A second linear mixed model compared the number of offspring produced by hybrids of *D. ananassae* and *D. pallidosa* when backcrossed to *D. ananassae* or *D. pallidosa* (Appendix B.). The reduced model provided no distinction in the class for the total number of offspring (Appendix C.). and the ANOVA tested the null hypothesis that the mean number of offspring produced by hybrids crossed to each of the parental species is not different.

A third linear mixed model compared the number of offspring produced by hybrid females compared to hybrid males (Appendix B.). The reduced model provided no distinction in the class for the total number of offspring (Appendix C.). The ANOVA tested the null hypothesis that there is no difference in the mean number of offspring produced by female hybrids and male hybrids.

I then used Coyne and Orr's isolation index to determine the minimum amount of postzygotic isolation between these populations (Coyne and Orr 1989). I assigned a value to every mating cross ranging from 0 to 1, where 0 indicates no isolation (every hybrid is viable), 1 indicates complete isolation (no hybrids are viable), and cases of Haldane's Rule are 0.5 (females produce offspring and males do not produce offspring). I considered a hybrid viable if any hybrid offspring survived to adulthood. The number of males and females produced by hybrids for each population comparison were averaged to arrive at a final score for each population cross. This method has also been used previously by Zouros (1973) and Throckmorton (1982).

CHAPTER III

RESULTS

I examined isofemale lines of *D. ananassae* originally collected from Trinity Beach, Australia Java, Indonesia, Nadi, Fiji, and Apia, Samoa. *D. pallidosa* were collected from Mallolelei, Samoa and Nadi, Fiji (Table 1). I performed 1193 individual crosses between *D. ananassae* and between *D. ananassae* and *D. pallidosa* and hybrids and 59 additional crosses between *D. ananassae* from Apia, Samoa and *D. pallidosa* from Mallolelei, Samoa. In all cases, number of offspring were counted as a measure of viability. I lumped the results into groups to test specific hypothesis about the degree of postzygotic isolation between species and between populations of *D. ananassae* as is described below.

Do *D. ananassae* and *D. pallidosa* have similar reproductive output?

I first determined whether there was any difference in the mean number of offspring produced by *D. ananassae* compared to *D. pallidosa* and found there is no significant difference (F= 3.0231, $d.f.$ = 4, P = 0.08208; Appendix C. Table 1C). The mean number of offspring produced by *D. ananassae* was 43.42±5.12 and the mean number of offspring produced by *D. pallidosa* was 30.52±4.42. The number of offspring produced on each date of the experiment varied considerably and using date as a random factor in the mixed linear model suggests that the variation contributed to by the date of

the crosses contributes enough to the difference in the mean number of offspring that the null hypothesis of no difference in the mean number of offspring produced by *D. ananassae* and *D. pallidosa* could not be rejected.

Do hybrid crosses between *D. ananassae* and *D. pallidosa* produce fewer offspring than each pure species cross?

Analysis of parental species: I found that there were very few crosses that produced either no males or no females (Table 4). The low percentage of crosses producing either no males, or no females indicates that there is no postzygotic isolation between *D. ananassae* and *D. pallidosa*. The results are consistent with Futch (1966).

Table 4: The percentage of reproductive success of hybrid crosses between *D. pallidosa* and *D. ananassae* in the F1 and F2 generation.

Generation	Male	Female	Percentage Producing Both Male and Female Offspring	Percentage Lacking Male Offspring	Percentage Lacking Female Offspring	Percentage Producing No Offspring
F1	*D. ananassae*	*D. pallidosa*	96%	2%	2%	0%
F1	*D. pallidosa*	*D. ananassae*	88%	9%	3%	0%
F1	*D. ananassae*	*D. ananassae*	97%	1%	1%	0%
F1	*D. pallidosa*	*D. pallidosa*	100%	0%	0%	0%
F2	*D. ananassae*	*D. ananassae*	98%	1%	0%	1%
F2	*D. pallidosa*	*D. pallidosa*	93%	3%	0%	3%
F2	*D. pallidosa* x *D. ananassae*	*D. pallidosa*	94%	6%	0%	0%
F2	*D. pallidosa* x *D. ananassae*	*D. ananassae*	92%	8%	0%	0%
F2	*D. ananassae* x *D. pallidosa*	*D. pallidosa*	100%	0%	0%	0%
F2	*D. ananassae* x *D. pallidosa*	*D. ananassae*	100%	0%	0%	0%
F2	*D. ananassae*	*D. pallidosa* x *D. ananassae*	100%	0%	0%	0%
F2	*D. pallidosa*	*D. pallidosa* x *D. ananassae*	96%	4%	0%	0%
F2	*D. ananassae*	*D. ananassae* x *D. pallidosa*	95%	0%	0%	5%
F2	*D. pallidosa*	*D. ananassae* x *D. pallidosa*	100%	0%	0%	0%

Analysis of hybrids: Hybrids produced by *D. ananassae* and *D. pallidosa* produced fewer offspring, on average (mean = 40.49±1.6792; Appendix D. Table 2D) than offspring from within species crosses (mean = 43.53±0.9583; $F = 10.675$, $d.f. = 4$, $P = 0.001$; Appendix C. Figure 2; Table 2C).

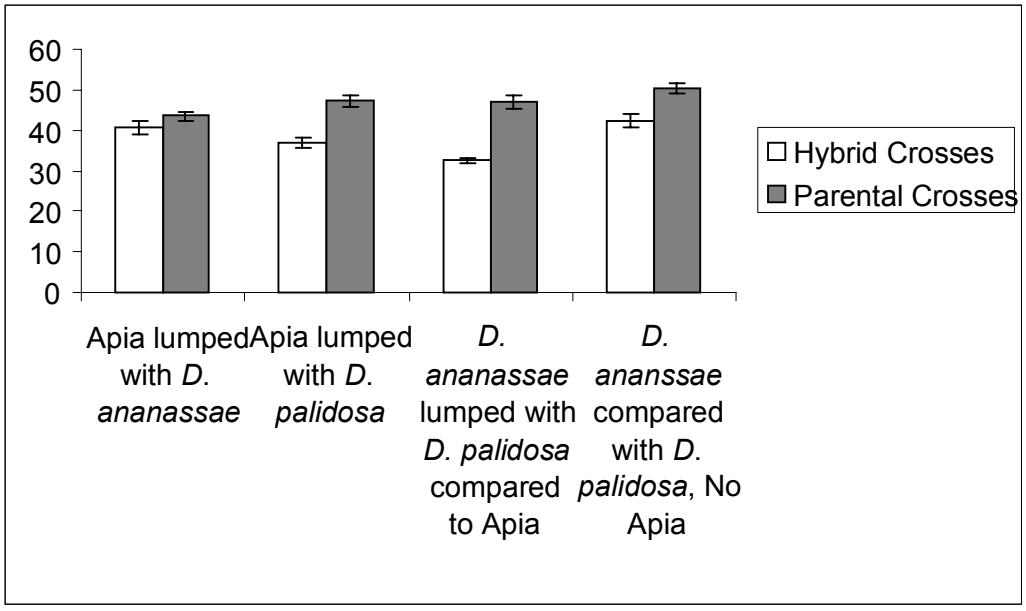

Figure 2: The number of offspring produced by hybrid offspring from *D. ananassae* and *D. pallidosa* crosses and offspring from within-species crosses. *D. ananassae* collected from Apia, Samoa were either lumped with *D. ananassae*, *D. pallidosa*, or treated as an independent species because of the uncertainty of their species status (Schug et al. 2008). The hybrid offspring from the between-species crosses produced fewer offspring regardless of the placement of the *D. ananassae* collected from Apia, Samoa than the offspring from the within-species crosses.

Analysis of hybrid backcrosses: There was no difference in the number of offspring produced when hybrids were backcrossed to *D. ananassae* or *D. pallidosa* ($F = 0.0131$, $d.f. = 4$, $P = 0.9088$; Appendix C, Table 2C). Further, there was no difference in the number of offspring produced by male hybrids backcrossed to parental types

compared to female hybrids backcrossed to parental types ($F = 2.5148$, $d.f. = 4$, $P = 0.1128$; Appendix C. Table 2C).

Analysis performed to disninguish Apia, Samoa population: Because the species status of the *D. ananassae* collected from Apia, Samoa is unclear (Schug et al. 2008); I also tested the same hypotheses by both lumping Apia *Drosophila* with *D. pallidosa* and by treating it as an independent species. I was motivated to explore this possibility because mitochondrial DNA analysis of the cytochrome b gene and control region (Smith 2005, Schug et al. 2008) demonstrated that the Apia, Samoa population was more distantly related to the other *D. ananassae* populations and *D. pallidosa* populations from Samoa and Fiji than the two species were to each other. Further, prezygotic isolation studies (Killon-Atwood 2005, Schug et al. 2008) demonstrated that *Drosophila* collected from Apia showed a high degree of mate discrimination with *D. ananassae* collected from Bogor, Indonesia, Malololelei, Samoa, Nadi, Fiji, and Trinity Beach, Australia.

I performed several tests using the viability data per cross from control and hybrid crosses: 1) Apia lumped with *D. ananassae* in comparison to *D. pallidosa* (the original comparison), 2) Apia lumped with *D. pallidosa* compared to *D. ananassae,* 3) *D. ananassae* compared to *D. pallidosa* removing Apia from the calculations, and 4) *D. pallidosa* lumped with *D. ananassae* and compared to Apia. The variance between the categories examined was low indicating a normal distribution (Appendix E.).

Analysis of parental species – Apia lumped with *D. ananassae*: As noted above, when the *D. ananassae* collected from Apia were lumped with the other *D.*

ananassae populations there were more offspring produced by offspring of the within-species crosses than hybrids but neither an effect caused by backcrossed to either *D. pallidosa* or *D. ananassae* nor an effect of hybrid gender was noted.

Analysis of parental species – Apia lumped with *D. pallidosa*: When *D. ananassae* collected from Apia were lumped with the *D. pallidosa* population, there were more offspring produced, on average, by the offspring of the within-species crosses (mean = 47.29±1.3758; Appendix D. Table 3D) than the hybrids (mean = 36.94±1.1011; $F = 82.722$, $d.f. = 4$, $P = 2.2 \times 10^{-16}$; Appendix C. Table 3C) but neither an effect caused by backcrossed to either *D. pallidosa* or *D. ananassae* ($F = 0.0005$, $d.f. = 4$, $P = 0.9822$; Appendix C, Table 3C.) nor a hybrid gender effect ($F = 3.6513$, $d.f. = 4$, $P = 0.05603$; Appendix C, Table 3C.) was noted.

Analysis of parental species – Apia removed from the analysis: When the *D. ananassae* collected from Apia were left out of the analysis, there were fewer offspring produced by hybrids (mean = 42.40±1.8344; Appendix D. Table 4D) relative to the number of offspring of the within-species crosses of *D. ananassae* and *D. pallidosa* (mean = 50.36±1.2237; $F = 25.855$, $d.f. = 4$, $P = 3.68 \times 10^{-7}$; Appendix C. Table 4C) but neither an effect caused by backcrossed to either *D. pallidosa* or *D. ananassae* ($F = 0.1621$, $d.f. = 4$, $P = 0.6872$; Appendix C, Table 4C.) nor a a hybrid gender effect ($F = 1.7479$, $d.f. = 4$, $P = 0.1861$; Appendix C, Table 4C.) was noted.

Analysis of parental species – Apia compared to *D. ananassae* lumped with *D. pallidosa*: When Apia was crossed to a lumped group of *D. ananassae* and *D. pallidosa* there were fewer offspring produced by the hybrids (32.58 ±0.7766; Appendix D. Table 5D) relative to the offspring of within-species crosses (mean = 47.07±1.6207; $F = 102.91$, $d.f. = 4$, $P = 2.2 \times 10^{-16}$; Appendix C. Table 5C) but neither an effect caused by backcrossed to either *D. pallidosa* or *D. ananassae* ($F = 1.8626$, $d.f. = 4$, $P = 0.1723$; Appendix C, Table 5C.) nor a hybrid gender effect ($F = 4.2121$, $d.f. = 4$, $P = 0.0401$; Appendix C, Table 5C.) was noted.

The results suggest that regardless of the species designation of Apia as *D. ananassae* or *D. pallidosa*, there is some reduction in the viability of the hybrid offspring produced by *D. ananassae and D. pallidosa*. However, the *Drosophila* collected from Apia, Samoa may have some added isolation caused by a lowered viability of hybrids when crossed to either *D. pallidosa* or *D. ananassae*. This reduction in viability is consistent with the hypothesis that *Drosophila* collected from Apia, Samoa are an independent species.

Do hybrids between *D. ananassae* from the South Pacific peripheral range and the Indonesian ancestral range show a reduced viability?

I tested the hypothesis that hybrids of the ancestral population (Bogor, Java; Vogl et al. 2002, Das et al. 2004) and South Pacific and Australian peripheral populations display reduced viability compared to the parental, within population crosses. These semi-isolated, peripheral populations that have been separated from each other for a

considerable amount of time by distance or other natural barriers to mating may have experienced adaptation to local ecological niches. These adaptations and the effects of genetic drift may have consequently increased hybrid inviability between populations. I focused on comparing viability between allopatric *D. ananassae* populations from Australia, Samoa, and Fiji relative to an ancestral population from Bogor, Java.

I expected that the closer a population is to Bogor, Java, the total number of offspring produced by hybrid crosses would more closely match the number of offspring produced by the parental types. This is because Bogor is the population deemed closest to the ancestral population (Vogl et al. 2004, Das et al. 2005, Smith 2006, Schug et al. 2007) while the other populations of *D. ananassae* and *D. palidosa* radiated from that geographic location to their respective locale, probably due to human interaction. If the *D. ananassae* radiated outward from Java with no human support, we would expect to see hybrid zones between the varied populations as if these populations were parapatric. Populations evolving via parapatric speciation are incompatible with each other between peripheral and the ancestral population, but do have hybrid zones between the two edges (Ridley 1996). However, if human movement were significant, I would expect to see the populations closest to human population centers to display less reproductive isolation than those populations with little human contact. The results of the number of offspring produced by hybrid of the ancestral versus peripheral population crosses are displayed in Figure 3.

Analyis of ancestral versus peripheral populations: There was no significant difference in the mean number of offspring produced by parental control crosses of *D.*

ananassae from Bogor, Java and the other South Pacific *D. ananassae* and *D. pallidosa* (Appendix D. Table 6D) and by hybrid crosses ($F = 1.6719$, $d.f. = 4$, $P = 0.203$; Appendix C. Table 6C). The difference in the mean number of offspring between parental control crosses of within-ancestral and within-peripheral populations (mean = 42.1±1.52) and hybrid crosses between ancestral and peripheral populations (mean = 45±1.0) seen in Figure 3 likely reflects the variance contributed by the dates the experiments were performed.

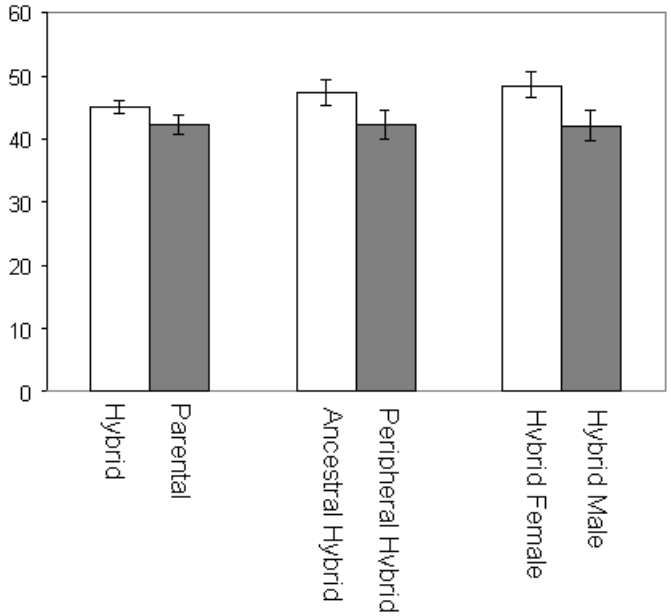

Figure 3*:* The number of offspring produced by offspring of within-species crosses and hybrid offspring from crosses between ancestral and peripheral populations of *D. ananassae*, the number of offspring produced by backcrosses of ancestral and peripheral hybrid males and females.

Analysis of ancestral/peripheral hybrids backcrossed to each parental

population: The direction of the backcross did not have an impact on the number of offspring produced (F =1.9799, *d.f.* = 4, P = 0.16; Appendix C. Table 6C). However, the male hybrids produced fewer offspring (mean = 42.0±2.4; Appendix D. Table 6D) than the female hybrids (mean = 48.47±2.01; F = 7.8025, *d.f.* = 4, P = 0.0052; Appendix C. Table 6C).

Do populations *of D. ananassae* and *D. pallidosa* that live in sympatry display greater postzygotic isolation than allopatric populations of *D. ananassae* and *D. pallidosa*?

Populations of *D. pallidosa* and *D. ananassae* in sympatry have been shown to display significant premating isolation (Killon-Atwood 2005, Schug et al. 2008). According to Futch (1966) and Orr (1989), premating isolation always occurs prior to postmating isolation in sympatric populations. Because premating isolation usually precedes postzygotic isolation and sympatric species display premating isolation prior to allopatric species, I tested the hypothesis that sympatric *D. ananassae* and *D. pallidosa* populations in Apia, Samoa show hybrid inviability.

To determine whether postzygotic isolation appears in sympatric species, *D. ananassae* from Apia, Samoa were crossed to *D. pallidosa* from Malololelei, Samoa. The distance between collection sites was within 10 km of each other and determined to be a continuous range (that is, there were no physical obstacles impeding free gamete exchange). Schug (pers. comm.) has collected *D. pallidosa* at low frequencies in Apia, the site where *D. ananassae* is most frequent.

Analysis of sympatric species hybrids: There was no significant difference in

the mean number of offspring produced (Figure 4) by Apia*, D. ananassae*/ Malololelei, *D. pallidosa* hybrids and pure species crosses ($F = 2.3079$, *d.f.* = 1, $P = 0.134$).

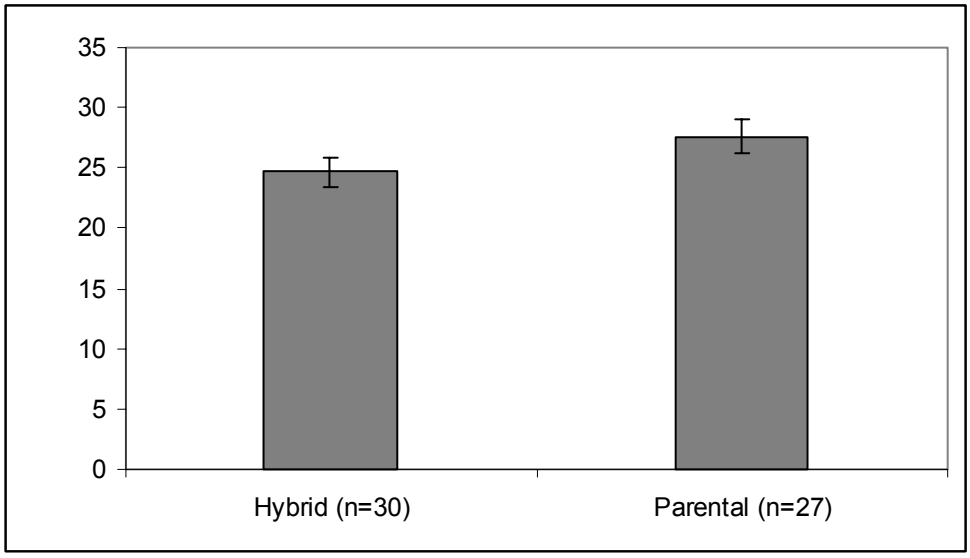

Figure 4: The average number of offspring produced by hybrids of *D. pallidosa* collected from Malololelei, Samoa and *D. ananassae* collected from Apia, Samoa. This figure shows that there is no significant difference between the number of offspring produced by parental and hybrid crosses.

Analysis of reciprocal crosses: The mean number of offspring from Malololelei males crossed to Apia females, and Apia males crossed to Malololelei females, were compared to pure species crosses to determine if there was an affect of sex on the number of offspring produced by *D. ananassae*/*D. pallidosa* hybrids in sympatry. These crosses were performed on the same day. Thus a simple ANOVA was conducted between the four possible crosses (Appendix F).

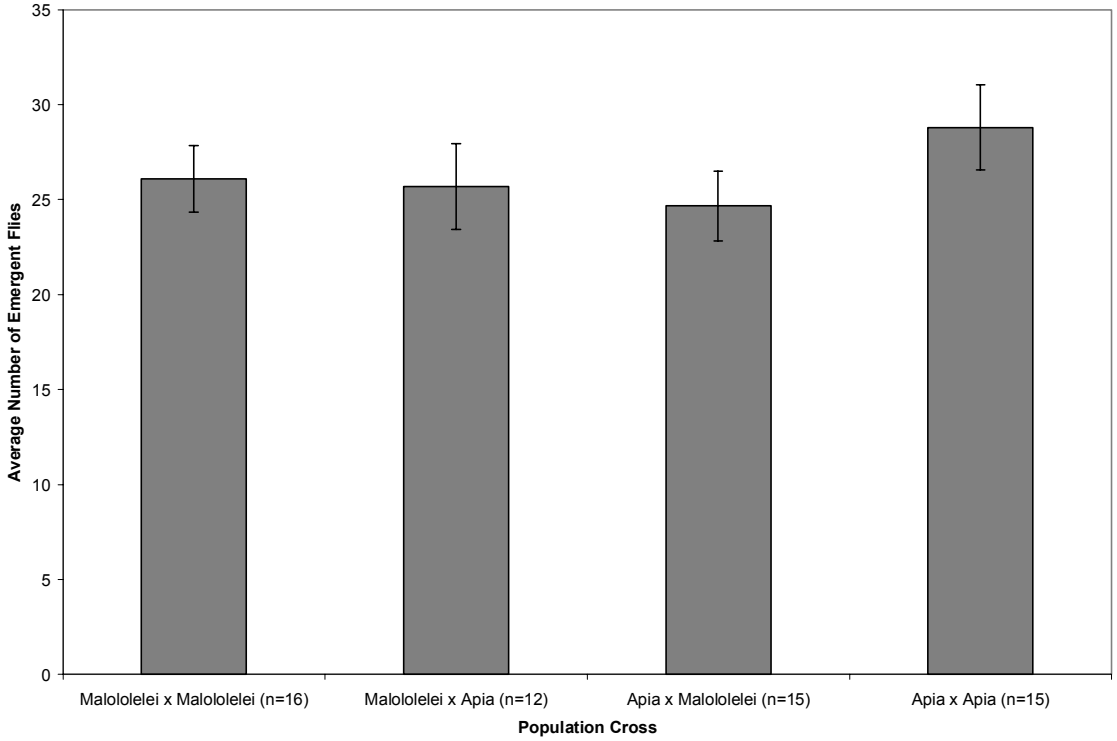

Figure 5: The average number of offspring produced by hybrids of *D. pallidosa* collected from Malololelei, Samoa and *D. ananassae* collected from Apia, Samoa. This figure shows how similar the number of offspring produced per cross of the parental crosses of these two populations are to the hybrid crosses.

There was no significant difference between the number of offspring produced by hybrids relative to within-species crosses (Figure 5; $F = 0.7463$, $d.f. = 3$, $P = 0.53$) indicating that there is no detectable hybrid inviability between sympatric populations of *D. ananassae* and *D. pallidosa* in Samoa. Thus, in Samoa sympatric populations of *D. ananassae* and *D. pallidosa* show no evidence of postzygotic sexual isolation.

Do the populations of *D. pallidosa* and *D. ananassae* examined in this study display postzygotic selection based on the Coyne and Orr (1989,1997) isolation index

Though I was primarily concerned with finding any measurable postzygotic isolation, Coyne and Orr (1989, 1997) argued that there can be no reproductive isolation unless either of the two sexes is sterile or inviable which they called the minimum amount of postzygotic isolation. Coyne and Orr proposed a mathematical method by which this isolation could be quantified (Coyne and Orr 1997). To determine the minimum amount of postzygotic selection between these populations, first I determined whether each cross between the populations was capable of producing both male and female offspring that could survive to adulthood and were fertile in the F1 generation (Table 5). I further determined whether the hybrid offspring produced from those crosses were reproductively compatible with the parental populations (Table 6).

TABLE 5: The percentage of reproductive success of hybrid crosses between populations of *D. pallidosa* and *D. ananassae* in the F1 Generation.

Male	Female	% Producing Both Male and Female Progeny	% Lacking Male or Female Progeny	% with No Progeny
Apia	Apia	88.24%	11.76%	0%
Apia	Bog	94.87%	5.12%	0%
Apia	Nadi	100%	0%	0%
Apia	Malololelei	100%	0%	0%
Apia	Pallidosa	100%	0%	0%
Apia	Trinity Beach	100%	0%	0%
Bog	Apia	100%	0%	0%
Bog	Bog	100%	0%	0%
Bog	Nadi	100%	0%	0%
Bog	Pallidosa	88.89%	11.11%	0%
Bog	Trinity Beach	100%	0%	0%
Nadi	Apia	100%	0%	0%
Nadi	Bog	100%	0%	0%
Nadi	Nadi	100%	0%	0%

Male	Female	Percentage Producing Both Male and Female Offspring	% Lacking Male or Female Progeny	% with No Progeny
Nadi	Pallidosa	100%	0%	0%
Nadi	Trinity Beach	100%	0%	0%
Pallidosa	Apia	100%	0%	0%
Pallidosa	Bog	85.71%	14.28%	0%
Pallidosa	Nadi	75.00%	25%	0%
Pallidosa	Pallidosa	100%	0%	0%
Pallidosa	Trinity Beach	100%	0%	0%
Malololelei	Apia	100%	0%	0%
Trinity Beach	Apia	100%	0%	0%
Trinity Beach	Bog	83.33%	16.67%	0%
Trinity Beach	Nadi	100%	0%	0%
Trinity Beach	Pallidosa	100%	0%	0%
Trinity Beach	Trinity Beach	100%	0%	0%

TABLE 6: The percentage of reproductive success of hybrid crosses between populations of *D. pallidosa* and *D. ananassae* in the F2 Generation.

Male	Female	% Producing Both Male and Female Progeny	% Lacking Male or Female Progeny	% with No Progeny
Apia	Apia-Apia	100%	0%	0%
Apia	Apia-Bog	95.83%	4.17%	0%
Apia	Apia-Nadi	100%	0%	0%
Apia	Apia-Pallidosa	100%	0%	0%
Apia	Apia-Trinity Beach	100%	0%	0%
Apia	Bog-Apia	100%	0%	0%
Apia	Nadi-Apia	100%	0%	0%
Apia	Pallidosa-Apia	100%	0%	0%
Apia	Trinity Beach-Apia	90%	10%	0%
Apia-Apia	Apia	100%	0%	0%
Apia-Bog	Apia	100%	0%	0%
Apia-Bog	Bog	100%	0%	0%
Apia-Nadi	Apia	100%	0%	0%
Apia-Nadi	Nadi	100%	0%	0%
Apia-Pallidosa	Apia	100%	0%	0%
Apia-Pallidosa	Pallidosa	100%	0%	0%
Apia-Trinity Beach	Apia	100%	0%	0%
Apia-Trinity Beach	Trinity Beach	100%	0%	0%
Bog	Apia-Bog	100%	0%	0%
Bog	Bog-Apia	100%	0%	0%
Bog	Bog-Bog	100%	0%	0%
Bog	Bog-Nadi	100%	0%	0%
Bog	Bog-Pallidosa	83.33%	0%	16.66%
Bog	Bog-Trinity Beach	100%	0%	0%
Bog	Nadi-Bog	100%	0%	0%
Bog	Pallidosa-Bog	100%	0%	0%
Bog	Trinity Beach-Bog	100%	0%	0%
Bog-Apia	Apia	100%	0%	0%
Bog-Apia	Bog	100%	0%	0%
Bog-Bog	Bog	100%	0%	0%
Bog-Nadi	Bog	85.71%	14.29%	0%
Bog-Nadi	Nadi	100%	0%	0%
Bog-Pallidosa	Bog	100%	0%	0%
Bog-Pallidosa	Pallidosa	100%	0%	0%
Bog-Trinity Beach	Bog	100%	0%	0%
Bog-Trinity Beach	Trinity Beach	100%	0%	0%
Nadi	Apia-Nadi	85.71%	14.29%	0%
Nadi	Bog-Nadi	66.67%	33.33%%	0%
Nadi	Nadi-Apia	100%	0%	0%
Nadi	Nadi-Bog	71.43%	28.57%	0%
Nadi	Nadi-Nadi	100%	0%	0%
Nadi	Nadi-Pallidosa	100%	0%	0%
Nadi	Nadi-Trinity Beach	90%	0%	10%
Nadi	Pallidosa-Nadi	100%	0%	0%
Nadi	Trinity Beach-Nadi	100%	0%	0%
Nadi-Apia	Apia	100%	0%	0%
Nadi-Apia	Nadi	100%	0%	0%
Nadi-Bog	Bog	100%	0%	0%
Nadi-Bog	Nadi	100%	0%	0%
Nadi-Nadi	Nadi	100%	0%	0%
Nadi-Pallidosa	Nadi	100%	0%	0%
Nadi-Pallidosa	Pallidosa	100%	0%	0%
Nadi-Trinity Beach	Nadi	100%	0%	0%
Nadi-Trinity Beach	Trinity Beach	100%	0%	0%
Pallidosa	Apia-Pallidosa	100%	0%	0%
Pallidosa	Bog-Pallidosa	100%	0%	0%
Pallidosa	Nadi-Pallidosa	100%	0%	0%
Pallidosa	Pallidosa-Apia	100%	0%	0%
Pallidosa	Pallidosa-Bog	100%	0%	0%
Pallidosa	Pallidosa-Nadi	80%	20%	0%
Pallidosa	Pallidosa-Pallidosa	100%	0%	0%
Pallidosa	Pallidosa-Trinity Beach	100%	0%	0%
Pallidosa	Trinity Beach-Pallidosa	100%	0%	0%
Pallidosa-Apia	Apia	100%	0%	0%
Pallidosa-Apia	Pallidosa	100%	0%	0%
Pallidosa-Bog	Bog	100%	0%	0%
Pallidosa-Bog	Pallidosa	100%	0%	0%
Pallidosa-Nadi	Pallidosa	81.82%	18.18%	0%
Pallidosa-Nadi	Nadi	66.67%	33.33%	0%
Pallidosa-Pallidosa	Pallidosa	88.24%	5.88%	6.67%
Pallidosa-Trinity Beach	Pallidosa	100%	0%	0%
Trinity Beach	Apia-Trinity Beach	100%	0%	0%
Trinity Beach	Bog-Trinity Beach	66.67%	0%	33.33%
Trinity Beach	Nadi-Trinity Beach	100%	0%	0%
Trinity Beach	Pallidosa-Trinity Beach	100%	0%	0%
Trinity Beach	Trinity Beach-Apia	94.44%	5.56%	0%
Trinity Beach	Trinity Beach-Bog	100%	0%	0%
Trinity Beach	Trinity-Beach-Nadi	100%	0%	0%
Trinity Beach	Trinity Beach-Pallidosa	100%	0%	0%
Trinity Beach	Trinity Beach-Trinity Beach	100%	0%	0%
Trinity Beach-Apia	Apia	100%	0%	0%
Trinity Beach-Apia	Trinity Beach	100%	0%	0%
Trinity Beach-Bog	Bog	95.45%	4.55%	0%
Trinity Beach-Bog	Trinity Beach	100%	0%	0%
Trinity-Beach-Nadi	Nadi	100%	0%	0%
Trinity-Beach-Nadi	Trinity Beach	100%	0%	0%
Trinity Beach-Pallidosa	Pallidosa	100%	0%	0%
Trinity Beach-Pallidosa	Trinity Beach	100%	0%	0%
Trinity Beach-Trinity Beach	Trinity Beach	100%	0%	0%

A value was assigned to ranging from 0 to 1 to each of these crosses where 0 indicates no isolation and 1 indicates complete isolation (cases of Haldane's Rule = 0.5). The minimum postzygotic isolation score for each population cross is shown in Figure 6.

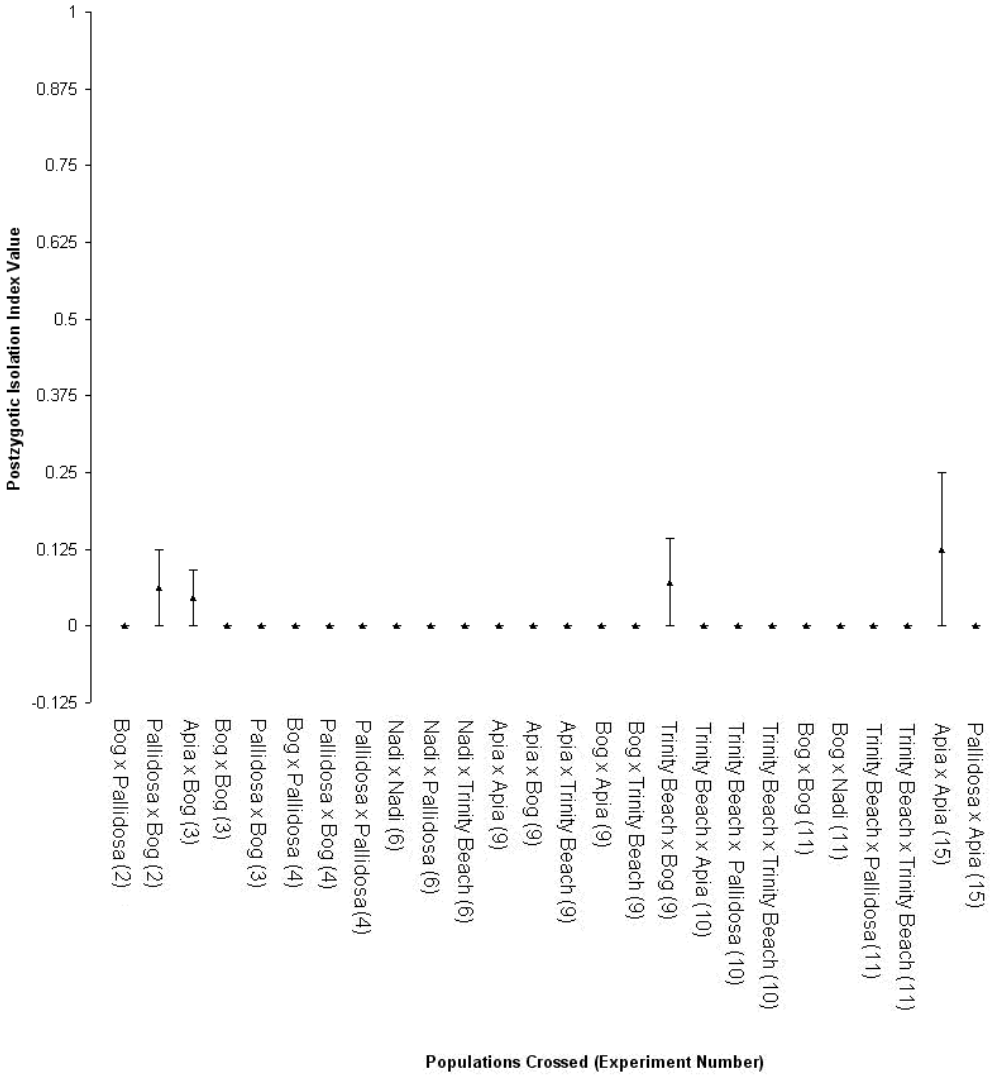

Figure 6: Minimum Postzygotic Isolation Index (calculated using the Coyne and Orr Method) among populations of *D. ananassae* and *D. pallidosa.* This figure demonstrates that there is no postzygotic isolation among these populations when quantified using Coyne and Orr's methodology.

The results indicated that most crosses scored a 0 (no isolation). Within some population crosses there were a minority of samples that produced either no male or no female offspring. These samples created a larger standard error within those population crosses than would be expected, but the values are still well below 0.5. This indicates that these populations do not show any postzygotic isolation using the Coyne and Orr isolation index. The standard error always includes a score of 0, indicating that there is no barrier to reproductive isolation.

Do semi-isolated populations of *Drosophila ananassae* display measurable pre-mating isolation prior to displaying measurable postzygotic reproductive isolation?

Coyne and Orr (1997) and Boake (2000) both presented evidence that in other species of *Drosophila* prezygotic isolation occurred prior to postzygotic isolation. Using the raw data from Killon-Atwood (2005), a pre-mating reproductive isolation index comparable to the postzygotic reproductive isolation index was established by subtracting the frequency of the heterospecific matings divided by the frequency of the homospecific matings in a multiple choice experiment from one (Table 7).

Adjusting the data to conform to the methods used by Coyne and Orr (1997), all of the possible population crosses rank a 0 on their scale. That is, all of the possible crosses between the populations are both fertile and viable. The results show that there is significant prezygotic isolation and no significant postzygotic isolation in any of the populations examined.

Table 7: The prezygotic and postzygotic isolation indexes created using the Coyne and Orr method based on the data from Killon-Atwood (2005) and this study.

Population 1	Population 2	Prezygotic Isolation Index (from Killon-Atwood 2005)	Postzygotic Isolation Index
Apia	Bog	0.3799	0
Apia	Nadi	incomplete	0
Apia	Pallidosa	1	0
Apia	Trinity Beach	0.687911343	0
Bog	Nadi	0.222222222	0
Bog	Pallidosa	0.491071429	0
Bog	Trinity Beach	0.344711538	0
Nadi	Pallidosa	0.895550528	0
Nadi	Trinity Beach	0.5575	0
Pallidosa	Trinity Beach	1	0

CHAPTER IV

DISCUSSION

In the early 1960s, experiments were conducted at the University of Texas studying the isolation of *D. ananassae* populations on South Pacific islands via genetic analysis, mate choice experiments, and measuring the viability of hybrid offspring (Futch 1966, Stone et al. 1966). The most advanced techniques in genetic analysis at the time consisted of cytology comparisons of the salivary glands and gel electrophoresis of isozymes. Several chromosomal inversions were identified between sympatric populations of *D. ananassae* in Samoa that displayed distinct morphological differences; a light form and a dark form (Futch 1966). These light and dark forms were then found to display complete mating discrimination when multiple-choice mating tests were conducted (Futch 1966). However, Futch noted that both hybrids of the light and dark forms are fertile. Stone et al. (1966) assayed the sympatric forms of *D. ananassae* for three components of fitness: the developmental viability of hybrids (how many survived to adulthood), fecundity (eggs laid per day), and fertility (number of fertile pairs). They found that the fertility and fecundity of the hybrid crosses varied widely and that the development viability of hybrids was more dependent on the degree of inbreeding than the type of cross. Based on the cytological and prezygotic isolation studies of Stone (1966) and Futch (1966) and by morphological differences examined by Bock and Wheeler (1972), the lighter form of *D. ananassae* was termed *D. pallidosa* regardless of

the lack of data on hybrid fitness.

Schug, McEvey, Smith, and Marshall collected *D. ananassae* and *D. pallidosa* from many of the same locations as Futch (1966) and Stone et al. (1966) during 2001-2003. More detailed genetic analyses of mtDNA and microsatellites were conducted to infer the level of genetic population structure. Their studies indicate that *D. ananassae* and *D. pallidosa* typically group as separate species but that there are some island populations that are more divergent from the two species than they are from each other (Smith 2005, Schug 2007, 2008). Trinity Beach, Australia and Apia, Samoa are two such populations. Killon-Atwood (2005) and Schug et al. (2008) performed multiple-choice mating experiments between *D. ananassae* and *D. pallidosa* populations from Indonesia, Australia, and the South Pacific. These studies indicated complete premating isolation between sympatric populations of *D. ananassae* and *D. pallidosa* in Samoa and Fiji (Killon-Atwood 2005, Schug 2008). These recent studies not only reinforced the findings of Futch (1966) and Stone (1966), but also suggested that Apia, Samoa and Trinity Beach, Australia populations may be incipient species. However, the findings of Killon-Atwood (2005), Smith (2005), and Schug et al. (2008) did not analyze the viability of any hybrid offspring.

Confounding effects of Experimental Date on Data.

I found that the date the individual experiments were conducted was a highly significant factor in the number of offspring produced per cross. *D. ananassae* are stenothermic, preferring a very specific set of environmental conditions that are difficult

to consistently reproduce in the lab (Tobari 1993). These may be the same conditions that Stone (1966) declares to be "uncontrolled laboratory conditions" affecting the outcome of his postzygotic reproduction experiments. To account for the contribution of date to the variance in the mean number of offspring where I performed experiments on different dates, I used a linear mixed model with date as a random effect to test the null hypothesis that there is no difference among the means of the number of offspring. In many cases, I was unable to reject the null hypothesis of no difference in the mean number of offspring produced by hybrids or hybrid backcrosses. Although the mixed linear model with date as a random factor accounts for the variance contributed to by the dates of the experiments, a more powerful analysis would be to conduct hybrid mating crosses on the same dates, and on the same batches of food as the crosses between individuals within each parental population.

Do *D. pallidosa* and *D. ananassae* show postzygotic sexual isolation?

The goal of my study was to determine the degree of postzygotic isolation among populations of *D. ananassae* and *D. pallidosa* in the same geographic range as those studied by Futch (1966), Stone et al. (1966), and Schug et al. (2007, 2008). I found that there is a measurable reduction in viability of hybrids of *D. ananassae* and *D. pallidosa* when mated in the laboratory. Further, I examined the viability of hybrids of the Bogor population compared to peripheral populations of *D. ananassae*. I found that there was no reduction in fitness between these two groups, consistent with Bogor, Java being within the ancestral species range, no effect caused by backcrossing the hybrids to either

the ancestral and peripheral populations parental types, and no effect of the hybrid sex on the number of offspring they produce. In addition, I found that sympatric populations of *D. ananassae* and *D. pallidosa* do not display any measurable postzygotic isolation in the F1 generation. Finally, I used an index of postzygotic mating isolation previously developed by Coyne and Orr (1989) to conservatively determine the rate at which prezygotic isolation evolves relative to postzygotic isolation and found that prezygotic isolation always evolves prior to postzygotic isolation in these populations of *D. ananassae* and *D. pallidosa.*

In my experiments, I measured the number offspring surviving to adulthood as a proxy of viability by counting the number of offspring produced by hybrids of crosses between populations of *D. ananassae* and hybrids of crosses between *D. pallidosa* and *D. ananassae*. I found that hybrids from crosses between species display a significant difference in viability consistent with the species status of *D. ananassae* and *D. pallidosa*, no difference in the number of offspring caused by the parental population to which the hybrid was crossed, and no difference in the number of offspring between male and female hybrids in crosses. Regardless of how the Apia, Samoa population was classified (as *D. pallidosa, D. ananassae*, or possibly its own species), the results of the crosses between *D. ananassae* and *D. pallidosa* consistently produced hybrids with lower viability, but no effect caused by the parental type when the hybrids were backcrossed nor an effect caused by the gender of the hybrid.

Do *D. ananassae* and *D. pallidosa* in sympatry show postzygotic sexual isolation?

Reproductive isolation may evolve based on environmental conditions (Funk

1998). One environmental condition that I was able to test was the effect that the presence of closely related species would have on viability. *D. ananassae* and *D. pallidosa* live in sympatry on Samoa. Sympatric populations of *Drosophila* have been shown to evolve prezygotic isolation with each other that often then leads to postzygotic isolation (Coyne and Orr 1997). However, even among these sympatric populations of *D. ananassae* and *D. pallidosa*, postzygotic isolation has yet to arise. Two population samples were examined in this study from Apia, Samoa and Malololelei, Samoa, both having an overlapping range of *D. ananassae* and *D. pallidosa*. Though collected from nearly the same location, the sympatric *Drosophila* displayed distinct morphological differences; those with a dark bodied form were believed to be *D. ananassae* while those with a light bodied form were termed *D. pallidosa.* Supporting this assertion were prezygotic sexual isolation studies that showed these populations display complete assortative mating (Killon-Atwood 2005, Schug et al. 2008). Because these populations were labeled as different species living in sympatry and they showed a high level of prezygotic sexual isolation, it was believed that they would have a high level of postzygotic isolation. This would serve to support the theory of speciation by reinforcement. The results of the cross between *D. ananassae* collected from Apia, Samoa and *D. pallidosa* collected from Malololelei, Samoa indicate that there is no postzygotic reproductive isolation occurring between these two populations. Neither inviability nor sterility appeared in this study. Additionally, because there was no difference between the number of offspring produced by backcrosses to either parental population nor was there a difference in the number of offspring produced by female

hybrids compared to male hybrids, Haldane's Rule and the Large X effect can be eliminated for considerations between these sympatric populations. Because the Malololelei and Apia populations displayed strong prezygotic isolation (Killon-Atwood 2005, Schug et al. 2008), this data serves as further evidence that prezygotic isolation develops prior to postzygotic isolation in sympatric populations. As such, it is still possible that at some future date true postzygotic isolation in the form of hybrid sterility or inviability will evolve between the two sympatric Samoan species prior to evolving between any of the allopatric populations.

Globally, however, my results indicate that if the ranges of *D. ananassae* and *D. pallidosa* were overlapping, hybrids would be selected against because they would produce fewer offspring than the parental types. This find is significant in that it supports mtDNA, microsatellite, and prezygotic isolation results (Smith 2005, Killon-Atwood 2005, Schug et al. 2007, Schug et al. 2008) suggesting that the *D. ananassae* and *D. pallidosa* populations are more related to each other than either is to the Apia, Samoa population.

Is the Apia, Samoa population an independent species?

It was suggested that the Apia, Samoa population may have colonized this geographic region within the last 5000 years (Schug et al. 2008) but recent studies have indicated that the population is an independent lineage of *D. ananassae* and may likely predate the split between the *D. ananassae* and *D. pallidosa* sister species (Schug et al. 2008). This long amount of time may have been enough to allow for co-adapted gene

complexes to form, isolating the Apia, Samoa population through pre-mating barriers (Killon-Atwood 2005, Schug et al. 2008) and also some post-mating barriers. The results lend support to the hypothesis that *D. ananassae* collected from Apia, Samoa are reproductively isolated from both *D. ananassae* and *D. pallidosa*. The Apia population has been shown to have a significant sexual isolation discrimination index with most of the other populations (Killon-Atwood 2005, Schug et al. 2008). Analysis of a phylogeny based on Mitochondrial DNA showed that Apia was more distantly related to the other populations than the *D. ananassae* collected from Trinity Beach, Bogor, Nadi, or the *D. pallidosa* collected from Nadi were to each other, suggesting that there is little historic gene flow between the *D. ananassae* collected from Apia, Samoa and the other populations (Smith 2005, Schug et al. 2007).

In my study, a reduction in hybrid viaility occurred when the Apia, Samoa population was compared to a lumping of *D. ananassae* and *D. pallidosa* collected from Indonesia, Australia, and Samoa. The reducation in hybrid viability was much greater than then reduction in viability between *D. pallidosa* and *D. ananassae* when the number of offspring produced by hybrids with the Apia, Samoa population were removed from the calculations. Because there was no difference in the number of offspring produced by hybrids backcrossed to either parental species nor a difference between the number of offspring produced by hybrid males compared to hybrid males, there is no indication that Haldane's Rule nor the Large X effect have become established between these groups. Regardless, the reduction in hybrid viability noted between the Apia population and a lumping of *D. ananassae* and *D. pallidosa* lends support to the theory that the Apia,

Samoa population may be more distantly related to *D. ananassae* and *D. pallidosa* populations than they are to each other.

Are peripheral populations of *D. ananassae* and *D. pallidosa* reproductively isolated from the proposed ancestral population of *D. ananassae*?

Reproductive isolation may evolve as a reflection of the amount of time that a population has been removed from the ancestral range of the species. I crossed *D. ananassae* from the ancestral range near Bogor, Java to populations from the peripheral range in the South Pacific and Australia. None of the hybrids from these crosses showed significant difference in viability, nor was there an overall reduction in hybrid viability between the ancestral and peripheral populations. Also, there was no difference in the number of offspring produced by hybrids backcrossed to either ancestral or peripheral parental groups indicating that there is no effect caused by backcrossing the hybrids of ancestral or peripheral populations to the parental types. Finally, there was no difference in the number of offspring produced by hybrid male crosses compared to hybrid female crosses indicating that Haldane's Rule and the Large X effect have not become established between the ancestral and peripheral populations. If *D. ananassae* radiated outward from Indonesia, they should not be as reproductively isolated from Bogor as they are from each other except for perhaps the extreme peripheral populations, much like European crows (Ridley 1996). My results are consistent with this hypothesis.

Is there any indication that prezygotic isolation may have evolved prior to postzygotic isolation?

Quantifying reproductive isolation in *D. ananassae* is difficult because minor variations in environmental conditions can have an enormous impact on the fitness (Stone et al. 1966). Coyne and Orr's Method of determining postzygotic isolation between species is a good indicator of whether two populations labeled as species follow the Biological Species Concept (Coyne and Orr 1989), but not whether there are minor variations in viability. In every scenario examined, there is no postzygotic isolation as described by Coyne and Orr (1989). Rather, *D. ananassae* is capable of producing fertile offspring with *D. pallidosa.* The appearance of both male and female offspring suggests that neither Haldane's Rule nor the Large X Effect is a factor in generating postzygotic isolation between these species. This method only describes strong postzygotic isolation and is not capable of examining smaller changes in viability that would indicate that a species is in the process of divergence.

To determine if small changes in viability of hybrids from population crosses have any measurable effect that could be used to determine if prezygotic isolation preceeds postzygotic isolation among *D. ananassae* and *D. pallidosa*, I used data from the from the Killon-Atwood (2005) mate choice studies. Clearly, the strong levels of prezygotic isolation in the Killon-Atwood (2005) and Schug et al. (2008) study, and the studies of Futch (1966) and Stone et al. (1966) suggest that behavioral differences among the populations have evolved and cause very strong prezygotic mating isolation. However, I was still interested in determining if the trends toward low levels of postzygotic isolation I documented may have preceeded the high levels of prezygotic isolation. The only method that has been proposed for examining this possibility is

Coyne and Orr's index of isolation. I used both the prezygotic isolation data from Killon-Atwood, and my postzygotic isolation data to determine the index of isolation using the Coyne and Orr method. The results show that prezygotic isolation always evolves prior to postzygotic isolation among these populations of *D. ananassae* and *D. pallidosa.* This is consistent with most theories concerning the relative speed at which isolation evolves in populations in which prezygotic isolation such as mating signals appear prior to postzygotic isolation mechanisms such as hybrid sterility (Coyne and Orr 1997, Boake 2000).

Beginning with Futch (1966) and Stone et al. (1966) and continuing through Killon-Atwood (2005), Smith (2005), and Schug et al. (2007, 2008) the characterization of *D. ananassae* as a model for incipent speciation has become clearer. Futch (1966) and Stone et al. (1966) described strong premating isolation between the light and dark forms of *D. ananassae* when given a choice as to mate and definitive differences in chromosome cytology but, due to uncontrolled laboratory conditions, that there was no postzygotic isolation found when the *D. ananassae* were given no choice of mate (Stone et al. 1966). Smith (2005), Killon-Atwood (2005), and Schug et al. (2007, 2008) reinforced the findings of Stone and Futch, adding that the Apia, Samoa population of *D. ananassae* may be in the process of speciation based on mitochondrial DNA, microsatellites, and mate choice studies and that Indonesia may be the ancestral range of *D. ananassae*. I have shown that there is a reduction in viability among hybrid offspring of *D. pallidosa* and *D. ananassae*, regardless of the species status of the Apia, Samoa population.

Trends indicating that hybrids are less viable than parental crosses between Apia, Samoa and each population are not significant which may be due to the confounding effects of the date of the experiment and small sample sizes. The potential prezygotic isolation between Apia and other *D. ananassae* populations should be explored further in controlled experiments.

I found that when *D. ananassae* and *D. pallidosa* are sympatric at a site with high levels of premating isolation, strong postmating isolation is not apparent. Further, the lack of premating isolation between the Indonesian population and peripheral populations supports Vogl (2003) in their assertion that Indonesia may be the ancestral range of *D. ananassae*. Finally, I compared the premating data of Killon-Atwood (2005) with the data I collected using Coyne and Orr's index of isolation (Coyne and Orr 1987) and found that premating isolation always occurs prior to postmating isolation among these populations. Based on genetic analyses, premating data, and postmating data, we can conclude that there is evidence that *D. pallidosa* and *D. ananassae* are different species as defined by the Biological Species Concept and that the Apia, Samoa population of *D. ananassae* may be independent species.

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Appendix A. Data Set for R Statistics Analysis

Legend: Apia = *D. ananassae* collected from Apia, Samoa; Bog = *D. ananassae* collected from Bogor, Java; TrinB = *D. ananassae* collected from Trinity Beach, Australia; Nadi = *D. ananassae* collected from Nadi, Fiji; Pallidosa = *D. pallidosa* collected from Nadi, Fiji; MaleP = collection site or crossing of the male parent (BogPal = a hybrid of male *D. ananassae* collected from Bogor, Java crossed to female *D. pallidosa* from Nadi, Fiji); FemaleP = collection site or crossing of the female parent; Pop = Population Description; Pop1 = Parental Viability vs Hybrid Viability; Pop2 = Hybrid Backcross to Parental Population; Pop3 = Male Hybrid Viability vs Female Hybrid Viability; Date = Experimental Date; TMal = Total Number of Male Offspring Produced; TFem = Total Number of Female Offspring Produced; TOS = Total Number of Offspring; SqRtTOS = Square Root Transformation of the Total Number of Offspring; PA = *D. ananassae* Parental Control Cross; PP = *D. pallidosa* Parental Control Cross; P = Parental; H= Hybrid; HA = Hybrid backcrossed to *D. ananassae*; HP = Hybrid Backcrossed to *D. pallidosa*; HM = Hybrid Male Backcrossed to Parental; HF = Hybrid Female Backcrossed to Parental

MaleP	FemP	Pop	Pop1	Pop2	Pop3	Date	TMal	TFem	TOS	SqRtTOS
BogBog	Bog	PA	P	P	P	5	38	33	71	8.43
BogBog	Bog	PA	P	P	P	5	22	21	43	6.56
BogBog	Bog	PA	P	P	P	5	33	25	58	7.62
BogBog	Bog	PA	P	P	P	5	28	29	57	7.55
BogBog	Bog	PA	P	P	P	5	35	33	68	8.25
BogBog	Bog	PA	P	P	P	5	40	33	73	8.54
BogBog	Bog	PA	P	P	P	5	28	44	72	8.49
BogBog	Bog	PA	P	P	P	5	42	31	73	8.54
BogBog	Bog	PA	P	P	P	5	33	31	64	8.00
BogBog	Bog	PA	P	P	P	5	36	47	83	9.11
BogBog	Bog	PA	P	P	P	5	38	33	71	8.43
BogBog	Bog	PA	P	P	P	5	22	21	43	6.56
BogBog	Bog	PA	P	P	P	5	33	25	58	7.62
BogBog	Bog	PA	P	P	P	5	28	29	57	7.55
BogBog	Bog	PA	P	P	P	5	35	33	68	8.25
BogBog	Bog	PA	P	P	P	5	40	33	73	8.54
BogBog	Bog	PA	P	P	P	5	28	44	72	8.49
BogBog	Bog	PA	P	P	P	5	42	31	73	8.54
BogBog	Bog	PA	P	P	P	5	33	31	64	8.00
BogBog	Bog	PA	P	P	P	5	36	47	83	9.11
BogBog	Bog	PA	P	P	P	5	38	33	71	8.43
BogBog	Bog	PA	P	P	P	5	22	21	43	6.56
BogBog	Bog	PA	P	P	P	5	33	25	58	7.62
BogBog	Bog	PA	P	P	P	5	28	29	57	7.55
BogBog	Bog	PA	P	P	P	5	35	33	68	8.25
BogBog	Bog	PA	P	P	P	5	40	33	73	8.54
BogBog	Bog	PA	P	P	P	5	28	44	72	8.49
BogBog	Bog	PA	P	P	P	5	42	31	73	8.54
BogBog	Bog	PA	P	P	P	5	33	31	64	8.00
BogBog	Bog	PA	P	P	P	5	36	47	83	9.11
BogBog	Bog	PA	P	P	P	5	38	33	71	8.43
BogBog	Bog	PA	P	P	P	5	22	21	43	6.56
BogBog	Bog	PA	P	P	P	5	33	25	58	7.62
BogBog	Bog	PA	P	P	P	5	28	29	57	7.55
BogBog	Bog	PA	P	P	P	5	35	33	68	8.25
BogBog	Bog	PA	P	P	P	5	40	33	73	8.54
BogBog	Bog	PA	P	P	P	5	28	44	72	8.49
BogBog	Bog	PA	P	P	P	5	42	31	73	8.54
BogBog	Bog	PA	P	P	P	5	33	31	64	8.00
BogBog	Bog	PA	P	P	P	5	36	47	83	9.11
BogTrinB	Bog	PA	P	P	P	7	32	39	71	8.43
BogTrinB	Bog	PA	P	P	P	7	24	21	45	6.71
BogTrinB	Bog	PA	P	P	P	7	27	16	43	6.56
BogTrinB	Bog	PA	P	P	P	7	18	21	39	6.24
BogTrinB	Bog	PA	P	P	P	7	11	24	35	5.92
BogTrinB	Bog	PA	P	P	P	7	18	14	32	5.66
BogTrinB	Bog	PA	P	P	P	7	6	14	20	4.47
BogTrinB	Bog	PA	P	P	P	7	21	23	44	6.63
TrinBApia	Apia	PA	P	P	P	7	3	3	6	2.45
TrinBApia	Apia	PA	P	P	P	7	7	5	12	3.46
TrinBApia	Apia	PA	P	P	P	7	6	10	16	4.00
TrinBApia	Apia	PA	P	P	P	7	2	2	4	2.00
TrinBApia	Apia	PA	P	P	P	7	5	0	5	2.24
TrinBApia	Apia	PA	P	P	P	7	2	8	10	3.16
TrinBApia	Apia	PA	P	P	P	7	3	1	4	2.00
TrinBBog	Bog	PA	P	P	P	7	8	10	18	4.24
TrinBBog	Bog	PA	P	P	P	7	23	15	38	6.16
TrinBBog	Bog	PA	P	P	P	7	12	12	24	4.90
TrinBBog	Bog	PA	P	P	P	7	0	5	5	2.24
TrinBBog	Bog	PA	P	P	P	7	6	6	12	3.46
TrinBBog	Bog	PA	P	P	P	7	34	24	58	7.62
TrinBBog	Bog	PA	P	P	P	7	5	7	12	3.46
TrinBBog	Bog	PA	P	P	P	7	31	30	61	7.81
TrinBBog	Bog	PA	P	P	P	7	8	18	26	5.10
TrinBBog	Bog	PA	P	P	P	7	24	14	38	6.16
TrinBBog	Bog	PA	P	P	P	7	11	7	18	4.24
TrinBBog	Bog	PA	P	P	P	7	5	6	11	3.32
TrinBBog	Bog	PA	P	P	P	7	15	12	27	5.20
ApiaBog	Apia	PA	P	P	P	8	18	16	34	5.83
ApiaBog	Apia	PA	P	P	P	8	28	17	45	6.71
ApiaBog	Apia	PA	P	P	P	8	8	9	17	4.12
ApiaBog	Apia	PA	P	P	P	8	18	23	41	6.40
ApiaBog	Apia	PA	P	P	P	8	8	9	17	4.12
ApiaBog	Apia	PA	P	P	P	8	9	7	16	4.00
ApiaBog	Apia	PA	P	P	P	8	19	21	40	6.32
ApiaBog	Apia	PA	P	P	P	8	15	11	26	5.10
ApiaBog	Apia	PA	P	P	P	8	16	6	22	4.69
ApiaBog	Apia	PA	P	P	P	8	19	11	30	5.48
ApiaTrinB	Apia	PA	P	P	P	10	8	13	21	4.58
ApiaTrinB	Apia	PA	P	P	P	10	24	11	35	5.92
ApiaTrinB	Apia	PA	P	P	P	10	19	11	30	5.48
ApiaTrinB	Apia	PA	P	P	P	10	14	24	38	6.16
ApiaTrinB	Apia	PA	P	P	P	10	14	15	29	5.39
NadiApia	Apia	PA	P	P	P	10	12	10	22	4.69
NadiApia	Apia	PA	P	P	P	10	14	16	30	5.48
NadiApia	Nadi	PA	P	P	P	10	11	3	14	3.74
NadiApia	Nadi	PA	P	P	P	10	21	14	35	5.92
NadiApia	Nadi	PA	P	P	P	10	12	18	30	5.48
NadiApia	Nadi	PA	P	P	P	10	30	10	40	6.32
NadiApia	Nadi	PA	P	P	P	10	1	3	4	2.00
NadiApia	Nadi	PA	P	P	P	10	4	3	7	2.65
NadiApia	Nadi	PA	P	P	P	10	1	1	2	1.41
NadiApia	Nadi	PA	P	P	P	10	2	5	7	2.65
NadiApia	Nadi	PA	P	P	P	10	15	11	26	5.10

NadiApia	Nadi	PA	P	P	P	10	10	19	29	5.39	ApiaBog	Bog	PA	P	P	P	12	8	8	16	4.00
NadiApia	Nadi	PA	P	P	P	10	6	13	19	4.36	ApiaTrinB	TrinB	PA	P	P	P	12	21	28	49	7.00
NadiApia	Nadi	PA	P	P	P	10	9	7	16	4.00	ApiaTrinB	TrinB	PA	P	P	P	12	8	11	19	4.36
NadiTrinB	Nadi	PA	P	P	P	10	18	18	36	6.00	ApiaTrinB	TrinB	PA	P	P	P	12	20	12	32	5.66
NadiTrinB	Nadi	PA	P	P	P	10	12	12	24	4.90	ApiaTrinB	TrinB	PA	P	P	P	12	4	7	11	3.32
NadiTrinB	Nadi	PA	P	P	P	10	3	2	5	2.24	BogApia	Apia	PA	P	P	P	12	1	1	2	1.41
TrinBNadi	Nadi	PA	P	P	P	10	18	19	37	6.08	BogApia	Apia	PA	P	P	P	12	17	11	28	5.29
TrinBNadi	Nadi	PA	P	P	P	10	23	14	37	6.08	BogApia	Apia	PA	P	P	P	12	13	5	18	4.24
TrinBNadi	Nadi	PA	P	P	P	10	50	48	98	9.90	BogApia	Apia	PA	P	P	P	12	8	8	16	4.00
TrinBNadi	Nadi	PA	P	P	P	10	34	29	63	7.94	BogApia	Bog	PA	P	P	P	12	18	14	32	5.66
TrinBNadi	TrinB	PA	P	P	P	10	54	70	124	11.14	BogApia	Bog	PA	P	P	P	12	12	8	20	4.47
TrinBNadi	TrinB	PA	P	P	P	10	48	67	115	10.72	BogApia	Bog	PA	P	P	P	12	13	18	31	5.57
TrinBNadi	TrinB	PA	P	P	P	10	33	39	72	8.49	BogApia	Bog	PA	P	P	P	12	20	16	36	6.00
TrinBNadi	TrinB	PA	P	P	P	10	42	47	89	9.43	BogApia	Bog	PA	P	P	P	12	24	21	45	6.71
TrinBNadi	TrinB	PA	P	P	P	10	82	69	151	12.29	BogApia	Bog	PA	P	P	P	12	13	14	27	5.20
TrinBNadi	TrinB	PA	P	P	P	10	51	41	92	9.59	BogApia	Bog	PA	P	P	P	12	5	4	9	3.00
TrinBNadi	TrinB	PA	P	P	P	10	33	28	61	7.81	BogApia	Bog	PA	P	P	P	12	23	13	36	6.00
ApiaApia	Apia	PA	P	P	P	12	11	14	25	5.00	BogApia	Bog	PA	P	P	P	12	13	19	32	5.66
ApiaApia	Apia	PA	P	P	P	12	7	2	9	3.00	BogApia	Bog	PA	P	P	P	12	18	17	35	5.92
ApiaApia	Apia	PA	P	P	P	12	9	7	16	4.00	BogApia	Bog	PA	P	P	P	12	27	31	58	7.62
ApiaApia	Apia	PA	P	P	P	12	1	6	7	2.65	BogApia	Bog	PA	P	P	P	12	30	25	55	7.42
ApiaApia	Apia	PA	P	P	P	12	1	5	6	2.45	BogApia	Bog	PA	P	P	P	12	19	19	38	6.16
ApiaApia	Apia	PA	P	P	P	12	11	14	25	5.00	BogNadi	Bog	PA	P	P	P	12	0	3	3	1.73
ApiaApia	Apia	PA	P	P	P	12	7	2	9	3.00	BogNadi	Bog	PA	P	P	P	12	5	6	11	3.32
ApiaApia	Apia	PA	P	P	P	12	9	7	16	4.00	BogNadi	Bog	PA	P	P	P	12	1	7	8	2.83
ApiaApia	Apia	PA	P	P	P	12	1	6	7	2.65	BogNadi	Bog	PA	P	P	P	12	6	5	11	3.32
ApiaApia	Apia	PA	P	P	P	12	1	5	6	2.45	BogNadi	Bog	PA	P	P	P	12	6	4	10	3.16
ApiaApia	Apia	PA	P	P	P	12	11	14	25	5.00	BogNadi	Bog	PA	P	P	P	12	2	3	5	2.24
ApiaApia	Apia	PA	P	P	P	12	7	2	9	3.00	BogNadi	Bog	PA	P	P	P	12	9	12	21	4.58
ApiaApia	Apia	PA	P	P	P	12	9	7	16	4.00	BogNadi	Nadi	PA	P	P	P	12	22	8	30	5.48
ApiaApia	Apia	PA	P	P	P	12	1	6	7	2.65	BogNadi	Nadi	PA	P	P	P	12	17	39	56	7.48
ApiaApia	Apia	PA	P	P	P	12	1	5	6	2.45	BogNadi	Nadi	PA	P	P	P	12	7	11	18	4.24
ApiaApia	Apia	PA	P	P	P	12	11	14	25	5.00	BogNadi	Nadi	PA	P	P	P	12	24	20	44	6.63
ApiaApia	Apia	PA	P	P	P	12	7	2	9	3.00	BogNadi	Nadi	PA	P	P	P	12	6	18	24	4.90
ApiaApia	Apia	PA	P	P	P	12	9	7	16	4.00	BogTrinB	TrinB	PA	P	P	P	12	26	21	47	6.86
ApiaApia	Apia	PA	P	P	P	12	1	6	7	2.65	BogTrinB	TrinB	PA	P	P	P	12	17	22	39	6.24
ApiaApia	Apia	PA	P	P	P	12	1	5	6	2.45	BogTrinB	TrinB	PA	P	P	P	12	25	28	53	7.28
ApiaBog	Bog	PA	P	P	P	12	19	13	32	5.66	BogTrinB	TrinB	PA	P	P	P	12	13	25	38	6.16
ApiaBog	Bog	PA	P	P	P	12	21	25	46	6.78	BogTrinB	TrinB	PA	P	P	P	12	33	30	63	7.94
ApiaBog	Bog	PA	P	P	P	12	5	5	10	3.16	BogTrinB	TrinB	PA	P	P	P	12	24	29	53	7.28
ApiaBog	Bog	PA	P	P	P	12	29	17	46	6.78	BogTrinB	TrinB	PA	P	P	P	12	35	31	66	8.12
ApiaBog	Bog	PA	P	P	P	12	6	4	10	3.16	BogTrinB	TrinB	PA	P	P	P	12	38	41	79	8.89
ApiaBog	Bog	PA	P	P	P	12	15	13	28	5.29	BogTrinB	TrinB	PA	P	P	P	12	36	29	65	8.06
ApiaBog	Bog	PA	P	P	P	12	25	19	44	6.63	NadiBog	Bog	PA	P	P	P	12	12	16	28	5.29
ApiaBog	Bog	PA	P	P	P	12	16	11	27	5.20	NadiBog	Bog	PA	P	P	P	12	17	20	37	6.08
ApiaBog	Bog	PA	P	P	P	12	10	11	21	4.58	NadiBog	Bog	PA	P	P	P	12	21	24	45	6.71
ApiaBog	Bog	PA	P	P	P	12	13	18	31	5.57	NadiBog	Bog	PA	P	P	P	12	16	22	38	6.16
ApiaBog	Bog	PA	P	P	P	12	12	12	24	4.90	NadiBog	Bog	PA	P	P	P	12	20	28	48	6.93

NadiBog	Bog	PA	P	P	P	12	14	14	28	5.29	ApiaNadi	Nadi	PA	P	P	P	15	26	35	61	7.81
NadiBog	Nadi	PA	P	P	P	12	18	14	32	5.66	ApiaNadi	Nadi	PA	P	P	P	15	13	17	30	5.48
NadiBog	Nadi	PA	P	P	P	12	15	12	27	5.20	NadiApia	Apia	PA	P	P	P	15	1	0	1	1.00
NadiBog	Nadi	PA	P	P	P	12	7	6	13	3.61	NadiApia	Apia	PA	P	P	P	15	26	19	45	6.71
NadiBog	Nadi	PA	P	P	P	12	11	7	18	4.24	NadiApia	Apia	PA	P	P	P	15	2	6	8	2.83
NadiTrinB	Nadi	PA	P	P	P	12	7	4	11	3.32	NadiApia	Apia	PA	P	P	P	15	18	18	36	6.00
NadiTrinB	Nadi	PA	P	P	P	12	11	14	25	5.00	NadiApia	Apia	PA	P	P	P	15	14	13	27	5.20
NadiTrinB	Nadi	PA	P	P	P	12	7	6	13	3.61	NadiApia	Apia	PA	P	P	P	15	16	8	24	4.90
NadiTrinB	Nadi	PA	P	P	P	12	18	9	27	5.20	NadiApia	Apia	PA	P	P	P	15	10	8	18	4.24
NadiTrinB	Nadi	PA	P	P	P	12	14	19	33	5.74	NadiApia	Apia	PA	P	P	P	15	9	13	22	4.69
NadiTrinB	Nadi	PA	P	P	P	12	13	10	23	4.80	NadiApia	Apia	PA	P	P	P	15	16	15	31	5.57
NadiTrinB	TrinB	PA	P	P	P	12	15	20	35	5.92	NadiApia	Apia	PA	P	P	P	15	9	8	17	4.12
NadiTrinB	TrinB	PA	P	P	P	12	14	17	31	5.57	NadiApia	Apia	PA	P	P	P	15	11	5	16	4.00
NadiTrinB	TrinB	PA	P	P	P	12	30	24	54	7.35	NadiApia	Apia	PA	P	P	P	15	5	4	9	3.00
NadiTrinB	TrinB	PA	P	P	P	12	39	31	70	8.37	NadiApia	Apia	PA	P	P	P	15	21	25	46	6.78
NadiTrinB	TrinB	PA	P	P	P	12	10	15	25	5.00	NadiNadi	Nadi	PA	P	P	P	15	8	6	14	3.74
TrinB	TrinB	PA	P	P	P	12	35	29	64	8.00	NadiNadi	Nadi	PA	P	P	P	15	24	18	42	6.48
TrinB	TrinB	PA	P	P	P	12	24	28	52	7.21	NadiNadi	Nadi	PA	P	P	P	15	27	19	46	6.78
TrinB	TrinB	PA	P	P	P	12	43	37	80	8.94	NadiNadi	Nadi	PA	P	P	P	15	21	28	49	7.00
TrinB	TrinB	PA	P	P	P	12	54	39	93	9.64	NadiNadi	Nadi	PA	P	P	P	15	8	6	14	3.74
TrinBTrinB	TrinB	PA	P	P	P	12	35	29	64	8.00	NadiNadi	Nadi	PA	P	P	P	15	24	18	42	6.48
TrinBTrinB	TrinB	PA	P	P	P	12	24	28	52	7.21	NadiNadi	Nadi	PA	P	P	P	15	27	19	46	6.78
TrinBTrinB	TrinB	PA	P	P	P	12	43	37	80	8.94	NadiNadi	Nadi	PA	P	P	P	15	21	28	49	7.00
TrinBTrinB	TrinB	PA	P	P	P	12	54	39	93	9.64	NadiNadi	Nadi	PA	P	P	P	15	8	6	14	3.74
TrinBTrinB	TrinB	PA	P	P	P	12	35	29	64	8.00	NadiNadi	Nadi	PA	P	P	P	15	24	18	42	6.48
TrinBTrinB	TrinB	PA	P	P	P	12	24	28	52	7.21	NadiNadi	Nadi	PA	P	P	P	15	27	19	46	6.78
TrinBTrinB	TrinB	PA	P	P	P	12	43	37	80	8.94	NadiNadi	Nadi	PA	P	P	P	15	21	28	49	7.00
TrinBTrinB	TrinB	PA	P	P	P	12	54	39	93	9.64	NadiNadi	Nadi	PA	P	P	P	15	8	6	14	3.74
TrinBTrinB	TrinB	PA	P	P	P	12	35	29	64	8.00	NadiNadi	Nadi	PA	P	P	P	15	24	18	42	6.48
TrinBTrinB	TrinB	PA	P	P	P	12	24	28	52	7.21	NadiNadi	Nadi	PA	P	P	P	15	27	19	46	6.78
TrinBTrinB	TrinB	PA	P	P	P	12	43	37	80	8.94	NadiNadi	Nadi	PA	P	P	P	15	21	28	49	7.00
TrinBTrinB	TrinB	PA	P	P	P	12	54	39	93	9.64	NadiTrinB	TrinB	PA	P	P	P	15	10	12	22	4.69
ApiaNadi	Apia	PA	P	P	P	15	8	15	23	4.80	NadiTrinB	TrinB	PA	P	P	P	15	31	31	62	7.87
ApiaNadi	Apia	PA	P	P	P	15	15	11	26	5.10	NadiTrinB	TrinB	PA	P	P	P	15	35	31	66	8.12
ApiaNadi	Apia	PA	P	P	P	15	15	19	34	5.83	NadiTrinB	TrinB	PA	P	P	P	15	51	46	97	9.85
ApiaNadi	Apia	PA	P	P	P	15	14	10	24	4.90	NadiTrinB	TrinB	PA	P	P	P	15	51	62	113	10.63
ApiaNadi	Apia	PA	P	P	P	15	15	13	28	5.29	NadiTrinB	TrinB	PA	P	P	P	15	53	84	137	11.70
ApiaNadi	Apia	PA	P	P	P	15	21	22	43	6.56	NadiTrinB	TrinB	PA	P	P	P	15	38	52	90	9.49
ApiaNadi	Apia	PA	P	P	P	15	14	18	32	5.66	NadiTrinB	TrinB	PA	P	P	P	15	40	39	79	8.89
ApiaNadi	Apia	PA	P	P	P	15	27	21	48	6.93	TrinBApia	TrinB	PA	P	P	P	15	13	9	22	4.69
ApiaNadi	Apia	PA	P	P	P	15	15	11	26	5.10	TrinBApia	TrinB	PA	P	P	P	15	55	37	92	9.59
ApiaNadi	Apia	PA	P	P	P	15	18	19	37	6.08	TrinBApia	TrinB	PA	P	P	P	15	48	54	102	10.10
ApiaNadi	Apia	PA	P	P	P	15	4	3	7	2.65	TrinBApia	TrinB	PA	P	P	P	15	6	20	26	5.10
ApiaNadi	Apia	PA	P	P	P	15	14	14	28	5.29	TrinBApia	TrinB	PA	P	P	P	15	53	62	115	10.72
ApiaNadi	Apia	PA	P	P	P	15	2	4	6	2.45	TrinBApia	TrinB	PA	P	P	P	15	26	27	53	7.28
ApiaNadi	Nadi	PA	P	P	P	15	4	4	8	2.83	TrinBApia	TrinB	PA	P	P	P	15	38	27	65	8.06
ApiaNadi	Nadi	PA	P	P	P	15	26	31	57	7.55	TrinBApia	TrinB	PA	P	P	P	15	29	37	66	8.12
ApiaNadi	Nadi	PA	P	P	P	15	27	31	58	7.62	TrinBApia	TrinB	PA	P	P	P	15	51	75	126	11.22

TrinBApia	TrinB	PA	P	P	P	15	31	51	82	9.06	NadiPal	Nadi	HmA	H	HA	hm	5	36	34	70	8.37
TrinBApia	TrinB	PA	P	P	P	15	45	55	100	10.00	NadiPal	Nadi	HmA	H	HA	hm	5	33	54	87	9.33
TrinBBog	Bog	PA	P	P	P	15	35	38	73	8.54	NadiPal	Nadi	HmA	H	HA	hm	5	39	48	87	9.33
TrinBBog	Bog	PA	P	P	P	15	47	76	123	11.09	NadiPal	Nadi	HmA	H	HA	hm	5	27	42	69	8.31
TrinBBog	Bog	PA	P	P	P	15	55	68	123	11.09	BogPal	Bog	HmA	H	HA	hm	6	8	5	13	3.61
TrinBBog	Bog	PA	P	P	P	15	52	53	105	10.25	BogPal	Bog	HmA	H	HA	hm	6	11	6	17	4.12
TrinBBog	Bog	PA	P	P	P	15	13	20	33	5.74	BogPal	Bog	HmA	H	HA	hm	6	1	4	5	2.24
TrinBBog	Bog	PA	P	P	P	15	34	50	84	9.17	BogPal	Bog	HmA	H	HA	hm	6	14	15	29	5.39
TrinBBog	Bog	PA	P	P	P	15	22	37	59	7.68	BogPal	Bog	HmA	H	HA	hm	6	10	11	21	4.58
TrinBBog	Bog	PA	P	P	P	15	65	76	141	11.87	BogPal	Bog	HmA	H	HA	hm	6	21	19	40	6.32
TrinBBog	Bog	PA	P	P	P	15	26	24	50	7.07	BogPal	Bog	HmA	H	HA	hm	6	27	22	49	7.00
TrinBBog	TrinB	PA	P	P	P	15	37	50	87	9.33	TrinBPal	TrinB	HmA	H	HA	hm	6	17	22	39	6.24
TrinBBog	TrinB	PA	P	P	P	15	24	37	61	7.81	TrinBPal	TrinB	HmA	H	HA	hm	6	8	11	19	4.36
TrinBBog	TrinB	PA	P	P	P	15	7	2	9	3.00	TrinBPal	TrinB	HmA	H	HA	hm	6	31	45	76	8.72
TrinBBog	TrinB	PA	P	P	P	15	24	36	60	7.75	TrinBPal	TrinB	HmA	H	HA	hm	6	35	38	73	8.54
TrinBBog	TrinB	PA	P	P	P	15	66	75	141	11.87	TrinBPal	TrinB	HmA	H	HA	hm	6	44	55	99	9.95
TrinBBog	TrinB	PA	P	P	P	15	68	45	113	10.63	TrinBPal	TrinB	HmA	H	HA	hm	6	4	6	10	3.16
TrinBBog	TrinB	PA	P	P	P	15	18	33	51	7.14	ApiaPal	Apia	HmA	H	HA	hm	12	33	23	56	7.48
TrinBBog	TrinB	PA	P	P	P	15	43	42	85	9.22	ApiaPal	Apia	HmA	H	HA	hm	12	16	28	44	6.63
TrinBBog	TrinB	PA	P	P	P	15	14	24	38	6.16	ApiaPal	Apia	HmA	H	HA	hm	12	33	23	56	7.48
TrinBBog	TrinB	PA	P	P	P	15	52	39	91	9.54	ApiaPal	Apia	HmA	H	HA	hm	12	45	33	78	8.83
TrinBBog	TrinB	PA	P	P	P	15	42	57	99	9.95	NadiPal	Pal	HmP	H	HP	hm	5	16	24	40	6.32
TrinBBog	TrinB	PA	P	P	P	15	53	44	97	9.85	NadiPal	Pal	HmP	H	HP	hm	5	39	28	67	8.19
TrinBBog	TrinB	PA	P	P	P	15	53	59	112	10.58	NadiPal	Pal	HmP	H	HP	hm	5	23	13	36	6.00
TrinBBog	TrinB	PA	P	P	P	15	43	51	94	9.70	NadiPal	Pal	HmP	H	HP	hm	5	27	30	57	7.55
BogPal	Bog	HmA	H	HA	hm	5	23	27	50	7.07	ApiaPal	Pal	HmP	H	HP	hm	6	18	13	31	5.57
BogPal	Bog	HmA	H	HA	hm	5	25	9	34	5.83	ApiaPal	Pal	HmP	H	HP	hm	6	6	9	15	3.87
BogPal	Bog	HmA	H	HA	hm	5	53	40	93	9.64	ApiaPal	Pal	HmP	H	HP	hm	6	7	11	18	4.24
BogPal	Bog	HmA	H	HA	hm	5	24	22	46	6.78	ApiaPal	Pal	HmP	H	HP	hm	6	1	1	2	1.41
BogPal	Bog	HmA	H	HA	hm	5	18	27	45	6.71	ApiaPal	Pal	HmP	H	HP	hm	6	23	24	47	6.86
BogPal	Bog	HmA	H	HA	hm	5	6	4	10	3.16	ApiaPal	Pal	HmP	H	HP	hm	6	9	15	24	4.90
BogPal	Bog	HmA	H	HA	hm	5	19	13	32	5.66	ApiaPal	Pal	HmP	H	HP	hm	6	8	11	19	4.36
BogPal	Bog	HmA	H	HA	hm	5	25	12	37	6.08	BogPal	Pal	HmP	H	HP	hm	6	8	5	13	3.61
BogPal	Bog	HmA	H	HA	hm	5	13	13	26	5.10	BogPal	Pal	HmP	H	HP	hm	6	11	6	17	4.12
BogPal	Bog	HmA	H	HA	hm	5	29	46	75	8.66	BogPal	Pal	HmP	H	HP	hm	6	1	4	5	2.24
BogPal	Bog	HmA	H	HA	hm	5	8	8	16	4.00	BogPal	Pal	HmP	H	HP	hm	6	14	15	29	5.39
BogPal	Bog	HmA	H	HA	hm	5	39	49	88	9.38	BogPal	Pal	HmP	H	HP	hm	6	10	11	21	4.58
BogPal	Bog	HmA	H	HA	hm	5	10	14	24	4.90	BogPal	Pal	HmP	H	HP	hm	6	21	19	40	6.32
BogPal	Bog	HmA	H	HA	hm	5	21	35	56	7.48	BogPal	Pal	HmP	H	HP	hm	6	27	22	49	7.00
BogPal	Bog	HmA	H	HA	hm	5	25	28	53	7.28	TrinBPal	Pal	HmP	H	HP	P	12	23	21	44	6.63
BogPal	Bog	HmA	H	HA	hm	5	38	34	72	8.49	TrinBPal	Pal	HmP	H	HP	P	12	18	21	39	6.24
BogPal	Bog	HmA	H	HA	hm	5	23	31	54	7.35	TrinBPal	Pal	HmP	H	HP	P	12	20	23	43	6.56
BogPal	Bog	HmA	H	HA	hm	5	37	26	63	7.94	TrinBPal	Pal	HmP	H	HP	P	12	27	47	74	8.60
BogPal	Bog	HmA	H	HA	hm	5	28	30	58	7.62	Apia	NadiApia	PA	P	P	P	5	32	37	69	8.31
BogPal	Bog	HmA	H	HA	hm	5	38	27	65	8.06	Apia	NadiApia	PA	P	P	P	5	28	33	61	7.81
BogPal	Bog	HmA	H	HA	hm	5	34	36	70	8.37	Apia	NadiApia	PA	P	P	P	5	29	21	50	7.07
BogPal	Bog	HmA	H	HA	hm	5	18	20	38	6.16	Apia	NadiApia	PA	P	P	P	5	6	3	9	3.00
BogPal	Bog	HmA	H	HA	hm	5	36	42	78	8.83	Bog	BogNadi	PA	P	P	P	6	35	30	65	8.06

Bog	BogNadi	PA	P	P	P	6	36	46	82	9.06	TrinB	TrinBApia	PA	P	P	P	6	3	9	12	3.46
Bog	BogNadi	PA	P	P	P	6	51	58	109	10.44	TrinB	TrinBApia	PA	P	P	P	6	1	1	2	1.41
Bog	BogNadi	PA	P	P	P	6	50	36	86	9.27	Apia	ApiaTrinB	PA	P	P	P	7	5	7	12	3.46
Bog	BogNadi	PA	P	P	P	6	29	22	51	7.14	Apia	ApiaTrinB	PA	P	P	P	7	6	8	14	3.74
Bog	BogNadi	PA	P	P	P	6	32	32	64	8.00	Apia	TrinBApia	PA	P	P	hm	7	10	6	16	4.00
Bog	BogNadi	PA	P	P	P	6	41	40	81	9.00	Apia	TrinBApia	PA	P	P	hm	7	6	7	13	3.61
Bog	BogNadi	PA	P	P	P	6	43	43	86	9.27	Apia	TrinBApia	PA	P	P	hm	7	14	10	24	4.90
Bog	BogNadi	PA	P	P	P	6	36	31	67	8.19	Apia	TrinBApia	PA	P	P	hm	7	3	5	8	2.83
Bog	BogNadi	PA	P	P	P	6	49	37	86	9.27	Apia	TrinBApia	PA	P	P	hm	7	0	2	2	1.41
Bog	BogNadi	PA	P	P	P	6	40	41	81	9.00	Apia	TrinBApia	PA	P	P	hm	7	10	3	13	3.61
Bog	BogNadi	PA	P	P	P	6	32	33	65	8.06	Apia	TrinBApia	PA	P	P	hm	7	20	16	36	6.00
Bog	BogNadi	PA	P	P	P	6	34	34	68	8.25	Apia	TrinBApia	PA	P	P	hm	7	0	0	0	0.00
Bog	BogNadi	PA	P	P	P	6	38	30	68	8.25	Apia	TrinBApia	PA	P	P	hm	7	4	12	16	4.00
Bog	BogNadi	PA	P	P	P	6	52	31	83	9.11	Apia	TrinBApia	PA	P	P	hm	7	5	5	10	3.16
Bog	NadiBog	PA	P	P	P	6	23	19	42	6.48	Apia	TrinBApia	PA	P	P	hm	7	3	6	9	3.00
Bog	NadiBog	PA	P	P	P	6	28	18	46	6.78	Bog	BogTrinB	PA	P	P	P	7	9	15	24	4.90
Bog	NadiBog	PA	P	P	P	6	19	29	48	6.93	Bog	BogTrinB	PA	P	P	P	7	22	46	68	8.25
Bog	NadiBog	PA	P	P	P	6	32	37	69	8.31	Bog	BogTrinB	PA	P	P	P	7	51	58	109	10.44
Bog	NadiBog	PA	P	P	P	6	19	13	32	5.66	Bog	BogTrinB	PA	P	P	P	7	46	38	84	9.17
Bog	NadiBog	PA	P	P	P	6	24	26	50	7.07	Bog	BogTrinB	PA	P	P	P	7	35	41	76	8.72
Bog	NadiBog	PA	P	P	P	6	36	34	70	8.37	Bog	TrinBBog	PA	P	P	P	7	35	33	68	8.25
Bog	NadiBog	PA	P	P	P	6	39	41	80	8.94	Bog	TrinBBog	PA	P	P	P	7	34	34	68	8.25
Bog	NadiBog	PA	P	P	P	6	37	19	56	7.48	Bog	TrinBBog	PA	P	P	P	7	44	40	84	9.17
Bog	NadiBog	PA	P	P	P	6	46	22	68	8.25	Bog	TrinBBog	PA	P	P	P	7	32	40	72	8.49
Bog	NadiBog	PA	P	P	P	6	56	43	99	9.95	Bog	TrinBBog	PA	P	P	P	7	35	28	63	7.94
Bog	NadiBog	PA	P	P	P	6	35	25	60	7.75	Apia	ApiaBog	PA	P	P	P	8	18	22	40	6.32
Bog	NadiBog	PA	P	P	P	6	44	29	73	8.54	Apia	ApiaBog	PA	P	P	P	8	0	3	3	1.73
Bog	NadiBog	PA	P	P	P	6	22	29	51	7.14	Apia	ApiaBog	PA	P	P	P	8	27	14	41	6.40
Bog	NadiBog	PA	P	P	P	6	42	29	71	8.43	Apia	ApiaBog	PA	P	P	P	8	14	11	25	5.00
Nadi	ApiaNadi	PA	P	P	P	6	0	3	3	1.73	Apia	ApiaBog	PA	P	P	P	8	11	8	19	4.36
Nadi	ApiaNadi	PA	P	P	P	6	23	29	52	7.21	Apia	ApiaBog	PA	P	P	P	8	19	6	25	5.00
Nadi	ApiaNadi	PA	P	P	P	6	13	33	46	6.78	Apia	ApiaBog	PA	P	P	P	8	27	33	60	7.75
Nadi	ApiaNadi	PA	P	P	P	6	1	1	2	1.41	Apia	ApiaBog	PA	P	P	P	8	27	16	43	6.56
Nadi	ApiaNadi	PA	P	P	P	6	6	10	16	4.00	Apia	ApiaBog	PA	P	P	P	8	14	18	32	5.66
Nadi	ApiaNadi	PA	P	P	P	6	31	17	48	6.93	Apia	ApiaBog	PA	P	P	P	8	8	8	16	4.00
Nadi	ApiaNadi	PA	P	P	P	6	21	23	44	6.63	Apia	ApiaBog	PA	P	P	P	8	12	10	22	4.69
TrinB	TrinBApia	PA	P	P	P	6	0	0	0	0.00	Apia	ApiaBog	PA	P	P	P	8	21	20	41	6.40
TrinB	TrinBApia	PA	P	P	P	6	8	9	17	4.12	Apia	ApiaBog	PA	P	P	P	8	18	16	34	5.83
TrinB	TrinBApia	PA	P	P	P	6	24	17	41	6.40	Apia	ApiaBog	PA	P	P	P	8	15	9	24	4.90
TrinB	TrinBApia	PA	P	P	P	6	6	3	9	3.00	Apia	ApiaBog	PA	P	P	P	8	16	19	35	5.92
TrinB	TrinBApia	PA	P	P	P	6	41	36	77	8.77	Apia	ApiaBog	PA	P	P	P	8	17	28	45	6.71
TrinB	TrinBApia	PA	P	P	P	6	23	21	44	6.63	Apia	ApiaBog	PA	P	P	P	8	23	25	48	6.93
TrinB	TrinBApia	PA	P	P	P	6	11	12	23	4.80	Apia	ApiaBog	PA	P	P	P	8	17	15	32	5.66
TrinB	TrinBApia	PA	P	P	P	6	8	11	19	4.36	Apia	ApiaBog	PA	P	P	P	8	28	31	59	7.68
TrinB	TrinBApia	PA	P	P	P	6	4	2	6	2.45	Apia	ApiaBog	PA	P	P	P	8	15	22	37	6.08
TrinB	TrinBApia	PA	P	P	P	6	3	9	12	3.46	Apia	ApiaBog	PA	P	P	P	8	23	27	50	7.07
TrinB	TrinBApia	PA	P	P	P	6	11	9	20	4.47	Apia	ApiaBog	PA	P	P	P	8	10	15	25	5.00
TrinB	TrinBApia	PA	P	P	P	6	6	12	18	4.24	Apia	ApiaBog	PA	P	P	P	8	17	26	43	6.56

Apia	ApiaBog	PA	P	P	P	8	1	2	3	1.73	Bog	BogBog	PA	P	P	P	8	32	30	62	7.87
Apia	ApiaBog	PA	P	P	P	8	25	30	55	7.42	Bog	BogBog	PA	P	P	P	8	39	33	72	8.49
Bog	BogBog	PA	P	P	P	8	33	36	69	8.31	Bog	BogBog	PA	P	P	P	8	32	27	59	7.68
Bog	BogBog	PA	P	P	P	8	22	36	58	7.62	Bog	BogBog	PA	P	P	P	8	31	23	54	7.35
Bog	BogBog	PA	P	P	P	8	25	47	72	8.49	Bog	BogBog	PA	P	P	P	8	29	26	55	7.42
Bog	BogBog	PA	P	P	P	8	23	20	43	6.56	Bog	BogBog	PA	P	P	P	8	33	36	69	8.31
Bog	BogBog	PA	P	P	P	8	34	48	82	9.06	Bog	BogBog	PA	P	P	P	8	22	36	58	7.62
Bog	BogBog	PA	P	P	P	8	24	22	46	6.78	Bog	BogBog	PA	P	P	P	8	25	47	72	8.49
Bog	BogBog	PA	P	P	P	8	26	20	46	6.78	Bog	BogBog	PA	P	P	P	8	23	20	43	6.56
Bog	BogBog	PA	P	P	P	8	43	36	79	8.89	Bog	BogBog	PA	P	P	P	8	34	48	82	9.06
Bog	BogBog	PA	P	P	P	8	28	43	71	8.43	Bog	BogBog	PA	P	P	P	8	24	22	46	6.78
Bog	BogBog	PA	P	P	P	8	41	28	69	8.31	Bog	BogBog	PA	P	P	P	8	26	20	46	6.78
Bog	BogBog	PA	P	P	P	8	45	26	71	8.43	Bog	BogBog	PA	P	P	P	8	43	36	79	8.89
Bog	BogBog	PA	P	P	P	8	42	43	85	9.22	Bog	BogBog	PA	P	P	P	8	28	43	71	8.43
Bog	BogBog	PA	P	P	P	8	32	30	62	7.87	Bog	BogBog	PA	P	P	P	8	41	28	69	8.31
Bog	BogBog	PA	P	P	P	8	39	33	72	8.49	Bog	BogBog	PA	P	P	P	8	45	26	71	8.43
Bog	BogBog	PA	P	P	P	8	32	27	59	7.68	Bog	BogBog	PA	P	P	P	8	42	43	85	9.22
Bog	BogBog	PA	P	P	P	8	31	23	54	7.35	Bog	BogBog	PA	P	P	P	8	32	30	62	7.87
Bog	BogBog	PA	P	P	P	8	29	26	55	7.42	Bog	BogBog	PA	P	P	P	8	39	33	72	8.49
Bog	BogBog	PA	P	P	P	8	33	36	69	8.31	Bog	BogBog	PA	P	P	P	8	32	27	59	7.68
Bog	BogBog	PA	P	P	P	8	22	36	58	7.62	Bog	BogBog	PA	P	P	P	8	31	23	54	7.35
Bog	BogBog	PA	P	P	P	8	25	47	72	8.49	Bog	BogBog	PA	P	P	P	8	29	26	55	7.42
Bog	BogBog	PA	P	P	P	8	23	20	43	6.56	Apia	ApiaNadi	PA	P	P	P	10	34	18	52	7.21
Bog	BogBog	PA	P	P	P	8	34	48	82	9.06	Apia	ApiaNadi	PA	P	P	P	10	27	17	44	6.63
Bog	BogBog	PA	P	P	P	8	24	22	46	6.78	Apia	ApiaNadi	PA	P	P	P	10	27	14	41	6.40
Bog	BogBog	PA	P	P	P	8	26	20	46	6.78	Apia	ApiaTrinB	PA	P	P	P	10	22	30	52	7.21
Bog	BogBog	PA	P	P	P	8	43	36	79	8.89	Apia	ApiaTrinB	PA	P	P	P	10	18	26	44	6.63
Bog	BogBog	PA	P	P	P	8	28	43	71	8.43	Apia	ApiaTrinB	PA	P	P	P	10	35	27	62	7.87
Bog	BogBog	PA	P	P	P	8	41	28	69	8.31	Apia	ApiaTrinB	PA	P	P	P	10	35	25	60	7.75
Bog	BogBog	PA	P	P	P	8	45	26	71	8.43	Apia	ApiaTrinB	PA	P	P	P	10	22	26	48	6.93
Bog	BogBog	PA	P	P	P	8	42	43	85	9.22	Nadi	NadiTrinB	PA	P	P	P	10	38	35	73	8.54
Bog	BogBog	PA	P	P	P	8	32	30	62	7.87	Nadi	NadiTrinB	PA	P	P	P	10	28	23	51	7.14
Bog	BogBog	PA	P	P	P	8	39	33	72	8.49	Nadi	NadiTrinB	PA	P	P	P	10	19	9	28	5.29
Bog	BogBog	PA	P	P	P	8	32	27	59	7.68	Nadi	NadiTrinB	PA	P	P	P	10	46	32	78	8.83
Bog	BogBog	PA	P	P	P	8	31	23	54	7.35	Nadi	NadiTrinB	PA	P	P	P	10	32	23	55	7.42
Bog	BogBog	PA	P	P	P	8	29	26	55	7.42	Nadi	NadiTrinB	PA	P	P	P	10	34	37	71	8.43
Bog	BogBog	PA	P	P	P	8	33	36	69	8.31	Nadi	TrinBNadi	PA	P	P	P	10	9	12	21	4.58
Bog	BogBog	PA	P	P	P	8	22	36	58	7.62	Nadi	TrinBNadi	PA	P	P	P	10	24	21	45	6.71
Bog	BogBog	PA	P	P	P	8	25	47	72	8.49	Nadi	TrinBNadi	PA	P	P	P	10	27	36	63	7.94
Bog	BogBog	PA	P	P	P	8	23	20	43	6.56	Nadi	TrinBNadi	PA	P	P	P	10	1	2	3	1.73
Bog	BogBog	PA	P	P	P	8	34	48	82	9.06	TrinB	ApiaTrinB	PA	P	P	P	10	32	26	58	7.62
Bog	BogBog	PA	P	P	P	8	24	22	46	6.78	TrinB	ApiaTrinB	PA	P	P	P	10	22	26	48	6.93
Bog	BogBog	PA	P	P	P	8	26	20	46	6.78	TrinB	ApiaTrinB	PA	P	P	P	10	10	13	23	4.80
Bog	BogBog	PA	P	P	P	8	43	36	79	8.89	TrinB	ApiaTrinB	PA	P	P	P	10	28	19	47	6.86
Bog	BogBog	PA	P	P	P	8	28	43	71	8.43	TrinB	ApiaTrinB	PA	P	P	P	10	38	43	81	9.00
Bog	BogBog	PA	P	P	P	8	41	28	69	8.31	TrinB	ApiaTrinB	PA	P	P	P	10	27	34	61	7.81
Bog	BogBog	PA	P	P	P	8	45	26	71	8.43	TrinB	ApiaTrinB	PA	P	P	P	10	41	31	72	8.49
Bog	BogBog	PA	P	P	P	8	42	43	85	9.22	TrinB	ApiaTrinB	PA	P	P	P	10	21	23	44	6.63

TrinB	ApiaTrinB	PA	P	P	P	10	17	22	39	6.24	Bog	ApiaBog	PA	P	P	P	12	22	22	44	6.63
TrinB	ApiaTrinB	PA	P	P	P	10	14	18	32	5.66	Bog	ApiaBog	PA	P	P	P	12	18	16	34	5.83
TrinB	ApiaTrinB	PA	P	P	P	10	16	13	29	5.39	Bog	ApiaBog	PA	P	P	P	12	17	15	32	5.66
TrinB	ApiaTrinB	PA	P	P	P	10	18	18	36	6.00	Bog	ApiaBog	PA	P	P	P	12	17	23	40	6.32
TrinB	ApiaTrinB	PA	P	P	P	10	12	11	23	4.80	Bog	ApiaBog	PA	P	P	P	12	14	12	26	5.10
TrinB	ApiaTrinB	PA	P	P	P	10	29	29	58	7.62	Bog	BogApia	PA	P	P	P	12	3	7	10	3.16
TrinB	ApiaTrinB	PA	P	P	P	10	25	45	70	8.37	Bog	BogApia	PA	P	P	P	12	21	29	50	7.07
TrinB	ApiaTrinB	PA	P	P	P	10	32	35	67	8.19	Bog	BogApia	PA	P	P	P	12	27	34	61	7.81
TrinB	ApiaTrinB	PA	P	P	P	10	4	2	6	2.45	Bog	BogApia	PA	P	P	P	12	20	35	55	7.42
TrinB	ApiaTrinB	PA	P	P	P	10	14	17	31	5.57	Bog	BogApia	PA	P	P	P	12	23	23	46	6.78
TrinB	ApiaTrinB	PA	P	P	P	10	24	23	47	6.86	Bog	BogApia	PA	P	P	P	12	10	20	30	5.48
TrinB	ApiaTrinB	PA	P	P	P	10	33	26	59	7.68	Bog	BogApia	PA	P	P	P	12	28	35	63	7.94
TrinB	ApiaTrinB	PA	P	P	P	10	14	11	25	5.00	Bog	BogApia	PA	P	P	P	12	27	32	59	7.68
TrinB	ApiaTrinB	PA	P	P	P	10	18	14	32	5.66	Bog	BogApia	PA	P	P	P	12	34	32	66	8.12
TrinB	ApiaTrinB	PA	P	P	P	10	20	24	44	6.63	Bog	BogApia	PA	P	P	P	12	19	13	32	5.66
TrinB	ApiaTrinB	PA	P	P	P	10	23	24	47	6.86	Bog	BogApia	PA	P	P	P	12	25	32	57	7.55
TrinB	TrinBNadi	PA	P	P	P	10	50	48	98	9.90	Nadi	BogNadi	PA	P	P	P	12	23	24	47	6.86
TrinB	TrinBNadi	PA	P	P	P	10	50	48	98	9.90	Nadi	BogNadi	PA	P	P	P	12	34	27	61	7.81
TrinB	TrinBNadi	PA	P	P	P	10	47	50	97	9.85	Nadi	BogNadi	PA	P	P	P	12	7	0	7	2.65
TrinB	TrinBNadi	PA	P	P	P	10	25	27	52	7.21	Nadi	NadiBog	PA	P	P	P	12	31	15	46	6.78
TrinB	TrinBNadi	PA	P	P	P	10	23	14	37	6.08	Nadi	NadiBog	PA	P	P	P	12	9	16	25	5.00
TrinB	TrinBNadi	PA	P	P	P	10	11	9	20	4.47	Nadi	NadiBog	PA	P	P	P	12	2	9	11	3.32
TrinB	TrinBNadi	PA	P	P	P	10	14	22	36	6.00	Nadi	NadiBog	PA	P	P	P	12	0	1	1	1.00
Apia	ApiaApia	PA	P	P	P	12	11	9	20	4.47	Nadi	NadiBog	PA	P	P	P	12	24	20	44	6.63
Apia	ApiaApia	PA	P	P	P	12	12	6	18	4.24	Nadi	NadiBog	PA	P	P	P	12	23	27	50	7.07
Apia	ApiaApia	PA	P	P	P	12	14	15	29	5.39	Nadi	NadiBog	PA	P	P	P	12	0	1	1	1.00
Apia	ApiaApia	PA	P	P	P	12	10	10	20	4.47	Nadi	NadiNadi	PA	P	P	P	12	25	30	55	7.42
Apia	ApiaApia	PA	P	P	P	12	11	9	20	4.47	Nadi	NadiNadi	PA	P	P	P	12	25	30	55	7.42
Apia	ApiaApia	PA	P	P	P	12	12	6	18	4.24	Nadi	NadiNadi	PA	P	P	P	12	25	30	55	7.42
Apia	ApiaApia	PA	P	P	P	12	14	15	29	5.39	Nadi	NadiTrinB	PA	P	P	P	12	23	24	47	6.86
Apia	ApiaApia	PA	P	P	P	12	10	10	20	4.47	Nadi	NadiTrinB	PA	P	P	P	12	3	3	6	2.45
Apia	ApiaApia	PA	P	P	P	12	11	9	20	4.47	Nadi	NadiTrinB	PA	P	P	P	12	0	0	0	0.00
Apia	ApiaApia	PA	P	P	P	12	12	6	18	4.24	Nadi	NadiTrinB	PA	P	P	P	12	19	21	40	6.32
Apia	ApiaApia	PA	P	P	P	12	14	15	29	5.39	Nadi	TrinBNadi	PA	P	P	P	12	1	3	4	2.00
Apia	ApiaApia	PA	P	P	P	12	10	10	20	4.47	Nadi	TrinBNadi	PA	P	P	P	12	7	11	18	4.24
Apia	ApiaApia	PA	P	P	P	12	11	9	20	4.47	TrinB	ApiaTrinB	PA	P	P	P	12	28	36	64	8.00
Apia	ApiaApia	PA	P	P	P	12	12	6	18	4.24	TrinB	ApiaTrinB	PA	P	P	P	12	26	21	47	6.86
Apia	ApiaApia	PA	P	P	P	12	14	15	29	5.39	TrinB	ApiaTrinB	PA	P	P	P	12	20	8	28	5.29
Apia	ApiaApia	PA	P	P	P	12	10	10	20	4.47	TrinB	ApiaTrinB	PA	P	P	P	12	22	11	33	5.74
Apia	BogApia	PA	P	P	P	12	5	8	13	3.61	TrinB	ApiaTrinB	PA	P	P	P	12	4	1	5	2.24
Apia	BogApia	PA	P	P	P	12	14	25	39	6.24	TrinB	BogTrinB	PA	P	P	P	12	30	26	56	7.48
Apia	BogApia	PA	P	P	P	12	19	13	32	5.66	TrinB	BogTrinB	PA	P	P	P	12	0	0	0	0.00
Apia	BogApia	PA	P	P	P	12	25	32	57	7.55	TrinB	BogTrinB	PA	P	P	P	12	5	7	12	3.46
Bog	ApiaBog	PA	P	P	P	12	33	25	58	7.62	TrinB	NadiTrinB	PA	P	P	P	12	16	12	28	5.29
Bog	ApiaBog	PA	P	P	P	12	12	18	30	5.48	TrinB	NadiTrinB	PA	P	P	P	12	15	10	25	5.00
Bog	ApiaBog	PA	P	P	P	12	11	21	32	5.66	TrinB	NadiTrinB	PA	P	P	P	12	27	16	43	6.56
Bog	ApiaBog	PA	P	P	P	12	28	20	48	6.93	TrinB	NadiTrinB	PA	P	P	P	12	21	18	39	6.24
Bog	ApiaBog	PA	P	P	P	12	26	19	45	6.71	TrinB	NadiTrinB	PA	P	P	P	12	28	24	52	7.21

TrinB	NadiTrinB	PA	P	P	P	12	20	25	45	6.71	Nadi	NadiNadi	PA	P	P	P	15	36	32	68	8.25
TrinB	NadiTrinB	PA	P	P	P	12	32	30	62	7.87	Nadi	NadiNadi	PA	P	P	P	15	25	28	53	7.28
TrinB	NadiTrinB	PA	P	P	P	12	25	31	56	7.48	Nadi	NadiNadi	PA	P	P	P	15	37	32	69	8.31
TrinB	NadiTrinB	PA	P	P	P	12	27	28	55	7.42	Nadi	NadiNadi	PA	P	P	P	15	40	29	69	8.31
TrinB	NadiTrinB	PA	P	P	P	12	29	24	53	7.28	Nadi	NadiNadi	PA	P	P	P	15	39	29	68	8.25
TrinB	NadiTrinB	PA	P	P	P	12	8	15	23	4.80	Nadi	NadiNadi	PA	P	P	P	15	36	32	68	8.25
TrinB	NadiTrinB	PA	P	P	P	12	11	8	19	4.36	Nadi	NadiNadi	PA	P	P	P	15	25	28	53	7.28
TrinB	NadiTrinB	PA	P	P	P	12	14	19	33	5.74	Nadi	NadiNadi	PA	P	P	P	15	37	32	69	8.31
TrinB	NadiTrinB	PA	P	P	P	12	14	15	29	5.39	Nadi	NadiNadi	PA	P	P	P	15	40	29	69	8.31
TrinB	NadiTrinB	PA	P	P	P	12	26	18	44	6.63	Nadi	NadiNadi	PA	P	P	P	15	39	29	68	8.25
TrinB	NadiTrinB	PA	P	P	P	12	29	29	58	7.62	Nadi	NadiNadi	PA	P	P	P	15	36	32	68	8.25
TrinB	Pal0	PA	P	P	P	12	43	37	80	8.94	Nadi	NadiNadi	PA	P	P	P	15	25	28	53	7.28
TrinB	Pal0	PA	P	P	P	12	42	26	68	8.25	Nadi	NadiNadi	PA	P	P	P	15	37	32	69	8.31
TrinB	Pal0	PA	P	P	P	12	6	15	21	4.58	Nadi	NadiNadi	PA	P	P	P	15	40	29	69	8.31
TrinB	Pal0	PA	P	P	P	12	20	23	43	6.56	Nadi	NadiNadi	PA	P	P	P	15	39	29	68	8.25
TrinB	TrinBTrinB	PA	P	P	P	12	43	37	80	8.94	TrinB	TrinBApia	PA	P	P	P	15	3	9	12	3.46
TrinB	TrinBTrinB	PA	P	P	P	12	42	26	68	8.25	TrinB	TrinBApia	PA	P	P	P	15	2	1	3	1.73
TrinB	TrinBTrinB	PA	P	P	P	12	6	15	21	4.58	TrinB	TrinBApia	PA	P	P	P	15	3	9	12	3.46
TrinB	TrinBTrinB	PA	P	P	P	12	20	23	43	6.56	TrinB	TrinBApia	PA	P	P	P	15	4	7	11	3.32
TrinB	TrinBTrinB	PA	P	P	P	12	43	37	80	8.94	TrinB	TrinBBog	PA	P	P	P	15	52	54	106	10.30
TrinB	TrinBTrinB	PA	P	P	P	12	42	26	68	8.25	TrinB	TrinBBog	PA	P	P	P	15	34	49	83	9.11
TrinB	TrinBTrinB	PA	P	P	P	12	6	15	21	4.58	TrinB	TrinBBog	PA	P	P	P	15	35	30	65	8.06
TrinB	TrinBTrinB	PA	P	P	P	12	20	23	43	6.56	TrinB	TrinBBog	PA	P	P	P	15	59	76	135	11.62
TrinB	TrinBTrinB	PA	P	P	P	12	43	37	80	8.94	TrinB	TrinBBog	PA	P	P	P	15	38	54	92	9.59
TrinB	TrinBTrinB	PA	P	P	P	12	42	26	68	8.25	TrinB	TrinBBog	PA	P	P	P	15	25	31	56	7.48
TrinB	TrinBTrinB	PA	P	P	P	12	6	15	21	4.58	TrinB	TrinBBog	PA	P	P	P	15	43	41	84	9.17
TrinB	TrinBTrinB	PA	P	P	P	12	20	23	43	6.56	TrinB	TrinBBog	PA	P	P	P	15	47	36	83	9.11
Apia	ApiaNadi	PA	P	P	P	15	26	13	39	6.24	TrinB	TrinBBog	PA	P	P	P	15	61	46	107	10.34
Apia	ApiaNadi	PA	P	P	P	15	11	15	26	5.10	Nadi	NadiPal	HfA	H	HA	hf	5	27	25	52	7.21
Apia	ApiaNadi	PA	P	P	P	15	3	6	9	3.00	Nadi	NadiPal	HfA	H	HA	hf	5	35	34	69	8.31
Apia	ApiaNadi	PA	P	P	P	15	7	13	20	4.47	Nadi	NadiPal	HfA	H	HA	hf	5	33	27	60	7.75
Apia	ApiaNadi	PA	P	P	P	15	22	15	37	6.08	Nadi	NadiPal	HfA	H	HA	hf	5	25	35	60	7.75
Apia	ApiaNadi	PA	P	P	P	15	14	14	28	5.29	Nadi	NadiPal	HfA	H	HA	hf	5	37	27	64	8.00
Apia	ApiaNadi	PA	P	P	P	15	23	23	46	6.78	Apia	ApiaPal	HfA	H	HA	hf	6	8	12	20	4.47
Apia	ApiaNadi	PA	P	P	P	15	25	15	40	6.32	Apia	ApiaPal	HfA	H	HA	hf	6	4	16	20	4.47
Bog	PalBog	PA	P	P	P	15	24	24	48	6.93	Apia	ApiaPal	HfA	H	HA	hf	6	34	37	71	8.43
Bog	PalBog	PA	P	P	P	15	10	7	17	4.12	Apia	ApiaPal	HfA	H	HA	hf	6	19	13	32	5.66
Bog	PalBog	PA	P	P	P	15	5	5	10	3.16	Bog	BogPal	HfA	H	HA	hf	6	11	7	18	4.24
Bog	PalBog	PA	P	P	P	15	18	16	34	5.83	Bog	BogPal	HfA	H	HA	hf	6	18	19	37	6.08
Nadi	NadiApia	PA	P	P	P	15	25	26	51	7.14	Bog	BogPal	HfA	H	HA	hf	6	11	19	30	5.48
Nadi	NadiApia	PA	P	P	P	15	23	15	38	6.16	Bog	BogPal	HfA	H	HA	hf	6	5	5	10	3.16
Nadi	NadiApia	PA	P	P	P	15	15	13	28	5.29	Bog	BogPal	HfA	H	HA	hf	6	0	0	0	0.00
Nadi	NadiApia	PA	P	P	P	15	19	21	40	6.32	Bog	BogPal	HfA	H	HA	hf	6	14	16	30	5.48
Nadi	NadiNadi	PA	P	P	P	15	36	32	68	8.25	Bog	BogPal	HfA	H	HA	hf	6	6	8	14	3.74
Nadi	NadiNadi	PA	P	P	P	15	25	28	53	7.28	TrinB	TrinBPal	HfA	H	HA	hf	10	25	34	59	7.68
Nadi	NadiNadi	PA	P	P	P	15	37	32	69	8.31	TrinB	TrinBPal	HfA	H	HA	hf	10	25	23	48	6.93
Nadi	NadiNadi	PA	P	P	P	15	40	29	69	8.31	TrinB	TrinBPal	HfA	H	HA	hf	10	27	30	57	7.55
Nadi	NadiNadi	PA	P	P	P	15	39	29	68	8.25	TrinB	PalTrinB	HfA	H	HA	hf	10	13	7	20	4.47

TrinB	PalTrinB	HfA	H	HA	hf	10	26	29	55	7.42	PalNadi	Pal	HmP	H	HP	hf	14	16	19	35	5.92
TrinB	PalTrinB	HfA	H	HA	hf	10	19	33	52	7.21	PalNadi	Pal	HmP	H	HP	hf	14	12	16	28	5.29
TrinB	PalTrinB	HfA	H	HA	hf	10	42	41	83	9.11	PalNadi	Pal	HmP	H	HP	hf	14	19	10	29	5.39
TrinB	PalTrinB	HfA	H	HA	hf	10	36	37	73	8.54	PalNadi	Pal	HmP	H	HP	hf	14	0	1	1	1.00
Nadi	PalNadi	HfA	H	HA	hf	14	9	16	25	5.00	PalNadi	Pal	HmP	H	HP	hf	14	8	15	23	4.80
Nadi	PalNadi	HfA	H	HA	hf	14	13	14	27	5.20	PalNadi	Pal	HmP	H	HP	hf	14	19	19	38	6.16
Nadi	PalNadi	HfA	H	HA	hf	14	5	9	14	3.74	PalBog	Pal	HmP	H	HP	hm	15	7	8	15	3.87
Nadi	PalNadi	HfA	H	HA	hf	14	4	9	13	3.61	PalBog	Pal	HmP	H	HP	hm	15	32	38	70	8.37
Apia	PalApia	HfA	H	HA	hf	15	6	8	14	3.74	PalBog	Pal	HmP	H	HP	hm	15	27	35	62	7.87
Apia	PalApia	HfA	H	HA	hf	15	6	8	14	3.74	PalPal	Pal	PP	P	P	P	14	18	21	39	6.24
Apia	PalApia	HfA	H	HA	hf	15	3	5	8	2.83	PalPal	Pal	PP	P	P	P	14	13	15	28	5.29
PalTrinB	TrinB	HmA	H	HA	hm	10	31	35	66	8.12	PalPal	Pal	PP	P	P	P	14	11	5	16	4.00
PalTrinB	TrinB	HmA	H	HA	hm	10	16	12	28	5.29	PalPal	Pal	PP	P	P	P	14	25	40	65	8.06
PalTrinB	TrinB	HmA	H	HA	hm	10	53	84	137	11.70	PalPal	Pal	PP	P	P	P	14	5	11	16	4.00
PalNadi	Pal	HmA	H	HA	hm	14	7	2	9	3.00	PalPal	Pal	PP	P	P	P	14	2	5	7	2.65
PalNadi	Pal	HmA	H	HA	hm	14	0	6	6	2.45	PalPal	Pal	PP	P	P	P	14	11	19	30	5.48
PalNadi	Pal	HmA	H	HA	hm	14	7	2	9	3.00	PalPal	Pal	PP	P	P	P	14	11	21	32	5.66
PalApia	Apia	HmA	H	HA	hm	15	26	24	50	7.07	PalPal	Pal	PP	P	P	P	14	14	14	28	5.29
PalApia	Apia	HmA	H	HA	hm	15	13	9	22	4.69	PalPal	Pal	PP	P	P	P	14	0	6	6	2.45
PalApia	Apia	HmA	H	HA	hm	15	6	6	12	3.46	PalPal	Pal	PP	P	P	P	14	19	20	39	6.24
PalBog	Bog	HmA	H	HA	hm	15	22	17	39	6.24	PalPal	Pal	PP	P	P	P	14	10	11	21	4.58
PalBog	Bog	HmA	H	HA	hm	15	22	16	38	6.16	PalPal	Pal	PP	P	P	P	14	21	24	45	6.71
PalBog	Bog	HmA	H	HA	hm	15	17	18	35	5.92	PalPal	Pal	PP	P	P	P	14	0	0	0	0.00
PalBog	Bog	HmA	H	HA	hm	15	19	21	40	6.32	PalPal	Pal	PP	P	P	P	14	3	1	4	2.00
PalApia	Pal	HmP	H	HP	hm	10	10	11	21	4.58	PalPal	Pal	PP	P	P	P	14	16	6	22	4.69
PalApia	Pal	HmP	H	HP	hm	10	4	3	7	2.65	PalPal	Pal	PP	P	P	P	14	48	38	86	9.27
PalApia	Pal	HmP	H	HP	hm	10	10	15	25	5.00	PalPal	Pal	PP	P	P	P	14	18	21	39	6.24
PalApia	Pal	HmP	H	HP	hm	10	2	19	21	4.58	PalPal	Pal	PP	P	P	P	14	13	15	28	5.29
PalTrinB	Pal	HmP	H	HP	hf	10	28	27	55	7.42	PalPal	Pal	PP	P	P	P	14	11	5	16	4.00
PalTrinB	Pal	HmP	H	HP	hf	10	5	5	10	3.16	PalPal	Pal	PP	P	P	P	14	25	40	65	8.06
PalTrinB	Pal	HmP	H	HP	hf	10	16	14	30	5.48	PalPal	Pal	PP	P	P	P	14	5	11	16	4.00
PalTrinB	Pal	HmP	H	HP	hf	10	23	25	48	6.93	PalPal	Pal	PP	P	P	P	14	2	5	7	2.65
PalTrinB	Pal	HmP	H	HP	hf	10	14	10	24	4.90	PalPal	Pal	PP	P	P	P	14	11	19	30	5.48
PalTrinB	Pal	HmP	H	HP	hf	10	23	25	48	6.93	PalPal	Pal	PP	P	P	P	14	11	21	32	5.66
PalTrinB	Pal	HmP	H	HP	hf	10	34	50	84	9.17	PalPal	Pal	PP	P	P	P	14	14	14	28	5.29
PalTrinB	Pal	HmP	H	HP	hf	10	23	24	47	6.86	PalPal	Pal	PP	P	P	P	14	0	6	6	2.45
PalTrinB	Pal	HmP	H	HP	hf	10	16	19	35	5.92	PalPal	Pal	PP	P	P	P	14	19	20	39	6.24
PalTrinB	Pal	HmP	H	HP	hf	10	9	7	16	4.00	PalPal	Pal	PP	P	P	P	14	10	11	21	4.58
PalTrinB	Pal	HmP	H	HP	hf	10	11	11	22	4.69	PalPal	Pal	PP	P	P	P	14	21	24	45	6.71
PalTrinB	Pal	HmP	H	HP	hf	10	12	12	24	4.90	PalPal	Pal	PP	P	P	P	14	0	0	0	0.00
PalTrinB	Pal	HmP	H	HP	hf	10	22	27	49	7.00	PalPal	Pal	PP	P	P	P	14	3	1	4	2.00
PalTrinB	Pal	HmP	H	HP	hf	10	29	22	51	7.14	PalPal	Pal	PP	P	P	P	14	16	6	22	4.69
PalTrinB	Pal	HmP	H	HP	hf	10	29	29	58	7.62	PalPal	Pal	PP	P	P	P	14	48	38	86	9.27
PalNadi	Pal	HmP	H	HP	hf	14	4	11	15	3.87	PalPal	Pal	PP	P	P	P	14	18	21	39	6.24
PalNadi	Pal	HmP	H	HP	hf	14	25	23	48	6.93	PalPal	Pal	PP	P	P	P	14	13	15	28	5.29
PalNadi	Pal	HmP	H	HP	hf	14	28	31	59	7.68	PalPal	Pal	PP	P	P	P	14	11	5	16	4.00
PalNadi	Pal	HmP	H	HP	hf	14	4	5	9	3.00	PalPal	Pal	PP	P	P	P	14	25	40	65	8.06
PalNadi	Pal	HmP	H	HP	hf	14	0	3	3	1.73	PalPal	Pal	PP	P	P	P	14	5	11	16	4.00

PalPal	Pal	PP	P	P	P	14	2	5	7	2.65	Pal	BogPal	HfP	H	HP	hf	8	24	25	49	7.00
PalPal	Pal	PP	P	P	P	14	11	19	30	5.48	Pal	BogPal	HfP	H	HP	hf	8	21	31	52	7.21
PalPal	Pal	PP	P	P	P	14	11	21	32	5.66	Pal	BogPal	HfP	H	HP	hf	8	15	18	33	5.74
PalPal	Pal	PP	P	P	P	14	14	14	28	5.29	Pal	BogPal	HfP	H	HP	hf	8	19	23	42	6.48
PalPal	Pal	PP	P	P	P	14	0	6	6	2.45	Pal	TrinBPal	HfP	H	HP	hf	10	41	34	75	8.66
PalPal	Pal	PP	P	P	P	14	19	20	39	6.24	Pal	TrinBPal	HfP	H	HP	hf	10	26	28	54	7.35
PalPal	Pal	PP	P	P	P	14	10	11	21	4.58	Pal	TrinBPal	HfP	H	HP	hf	10	28	26	54	7.35
PalPal	Pal	PP	P	P	P	14	21	24	45	6.71	Pal	BogPal	HfP	H	HP	hf	15	8	7	15	3.87
PalPal	Pal	PP	P	P	P	14	0	0	0	0.00	Pal	BogPal	HfP	H	HP	hf	15	28	24	52	7.21
PalPal	Pal	PP	P	P	P	14	3	1	4	2.00	Pal	BogPal	HfP	H	HP	hf	15	7	10	17	4.12
PalPal	Pal	PP	P	P	P	14	16	6	22	4.69	Pal	BogPal	HfP	H	HP	hf	15	28	25	53	7.28
PalPal	Pal	PP	P	P	P	14	48	38	86	9.27	Pal	BogPal	HfP	H	HP	hf	15	16	14	30	5.48
PalPal	Pal	PP	P	P	P	14	18	21	39	6.24	Pal	PalTrinB	HfP	H	HP	hf	10	30	11	41	6.40
PalPal	Pal	PP	P	P	P	14	13	15	28	5.29	Pal	PalTrinB	HfP	H	HP	hf	10	9	11	20	4.47
PalPal	Pal	PP	P	P	P	14	11	5	16	4.00	Pal	PalTrinB	HfP	H	HP	hf	10	12	19	31	5.57
PalPal	Pal	PP	P	P	P	14	25	40	65	8.06	Pal	PalTrinB	HfP	H	HP	hf	10	12	20	32	5.66
PalPal	Pal	PP	P	P	P	14	5	11	16	4.00	Pal	PalTrinB	HfP	H	HP	hf	10	32	24	56	7.48
PalPal	Pal	PP	P	P	P	14	2	5	7	2.65	Pal	PalTrinB	HfP	H	HP	hf	10	18	19	37	6.08
PalPal	Pal	PP	P	P	P	14	11	19	30	5.48	Pal	PalTrinB	HfP	H	HP	hf	10	22	29	51	7.14
PalPal	Pal	PP	P	P	P	14	11	21	32	5.66	Pal	PalTrinB	HfP	H	HP	hf	10	30	27	57	7.55
PalPal	Pal	PP	P	P	P	14	14	14	28	5.29	Pal	PalTrinB	HfP	H	HP	hf	10	29	44	73	8.54
PalPal	Pal	PP	P	P	P	14	0	6	6	2.45	Pal	PalTrinB	HfP	H	HP	hf	10	7	16	23	4.80
PalPal	Pal	PP	P	P	P	14	19	20	39	6.24	Pal	PalNadi	HfP	H	HP	hf	14	12	16	28	5.29
PalPal	Pal	PP	P	P	P	14	10	11	21	4.58	Pal	PalNadi	HfP	H	HP	hf	14	19	10	29	5.39
PalPal	Pal	PP	P	P	P	14	21	24	45	6.71	Pal	PalNadi	HfP	H	HP	hf	14	0	1	1	1.00
PalPal	Pal	PP	P	P	P	14	0	0	0	0.00	Pal	PalNadi	HfP	H	HP	hf	14	8	15	23	4.80
PalPal	Pal	PP	P	P	P	14	3	1	4	2.00	Pal	PalNadi	HfP	H	HP	hf	14	19	19	38	6.16
PalPal	Pal	PP	P	P	P	14	16	6	22	4.69	Pal	PalApia	HfP	H	HP	hf	15	15	11	26	5.10
PalPal	Pal	PP	P	P	P	14	48	38	86	9.27	Pal	PalApia	HfP	H	HP	hf	15	18	19	37	6.08
Pal	ApiaPal	HfP	H	HP	hf	6	11	19	30	5.48	Pal	PalApia	HfP	H	HP	hf	15	4	3	7	2.65
Pal	ApiaPal	HfP	H	HP	hf	6	20	12	32	5.66	Pal	PalApia	HfP	H	HP	hf	15	14	14	28	5.29
Pal	ApiaPal	HfP	H	HP	hf	6	21	14	35	5.92	Pal	PalApia	HfP	H	HP	hf	15	2	4	6	2.45
Pal	NadiPal	HfP	H	HP	hf	6	30	40	70	8.37	Pal	PalBog	HfP	H	HP	hf	15	11	13	24	4.90
Pal	NadiPal	HfP	H	HP	hf	6	25	38	63	7.94	Pal	PalBog	HfP	H	HP	hf	15	24	24	48	6.93
Pal	NadiPal	HfP	H	HP	hf	6	42	41	83	9.11	Pal	PalBog	HfP	H	HP	hf	15	28	18	46	6.78
Pal	NadiPal	HfP	H	HP	hf	6	31	34	65	8.06	Pal	PalPal	PP	P	P	P	14	17	14	31	5.57
Pal	NadiPal	HfP	H	HP	hf	6	7	11	18	4.24	Pal	PalPal	PP	P	P	P	14	19	17	36	6.00
Pal	NadiPal	HfP	H	HP	hf	6	41	51	92	9.59	Pal	PalPal	PP	P	P	P	14	19	18	37	6.08
Pal	NadiPal	HfP	H	HP	hf	6	21	37	58	7.62	Pal	PalPal	PP	P	P	P	14	8	6	14	3.74
Pal	NadiPal	HfP	H	HP	hf	6	20	19	39	6.24	Pal	PalPal	PP	P	P	P	14	22	10	32	5.66
Pal	NadiPal	HfP	H	HP	hf	6	10	17	27	5.20	Pal	PalPal	PP	P	P	P	14	2	3	5	2.24
Pal	NadiPal	HfP	H	HP	hf	6	27	23	50	7.07	Pal	PalPal	PP	P	P	P	14	16	22	38	6.16
Pal	NadiPal	HfP	H	HP	hf	6	29	35	64	8.00	Pal	PalPal	PP	P	P	P	14	19	24	43	6.56
Pal	NadiPal	HfP	H	HP	hf	6	38	27	65	8.06	Pal	PalPal	PP	P	P	P	14	9	9	18	4.24
Pal	NadiPal	HfP	H	HP	hf	6	27	23	50	7.07	Pal	PalPal	PP	P	P	P	14	14	14	28	5.29
Pal	NadiPal	HfP	H	HP	hm	6	37	41	78	8.83	Pal	PalPal	PP	P	P	P	14	8	12	20	4.47
Pal	BogPal	HfP	H	HP	hf	8	16	12	28	5.29	Pal	PalPal	PP	P	P	P	14	15	20	35	5.92
Pal	BogPal	HfP	H	HP	hf	8	18	24	42	6.48	Pal	PalPal	PP	P	P	P	14	1	9	10	3.16

Pal	PalPal	PP	P	P	P	14	17	14	31	5.57
Pal	PalPal	PP	P	P	P	14	19	17	36	6.00
Pal	PalPal	PP	P	P	P	14	19	18	37	6.08
Pal	PalPal	PP	P	P	P	14	8	6	14	3.74
Pal	PalPal	PP	P	P	P	14	22	10	32	5.66
Pal	PalPal	PP	P	P	P	14	2	3	5	2.24
Pal	PalPal	PP	P	P	P	14	16	22	38	6.16
Pal	PalPal	PP	P	P	P	14	19	24	43	6.56
Pal	PalPal	PP	P	P	P	14	9	9	18	4.24
Pal	PalPal	PP	P	P	P	14	14	14	28	5.29
Pal	PalPal	PP	P	P	P	14	8	12	20	4.47
Pal	PalPal	PP	P	P	P	14	15	20	35	5.92
Pal	PalPal	PP	P	P	P	14	1	9	10	3.16
Pal	PalPal	PP	P	P	P	14	17	14	31	5.57
Pal	PalPal	PP	P	P	P	14	19	17	36	6.00
Pal	PalPal	PP	P	P	P	14	19	18	37	6.08
Pal	PalPal	PP	P	P	P	14	8	6	14	3.74
Pal	PalPal	PP	P	P	P	14	22	10	32	5.66
Pal	PalPal	PP	P	P	P	14	2	3	5	2.24
Pal	PalPal	PP	P	P	P	14	16	22	38	6.16
Pal	PalPal	PP	P	P	P	14	19	24	43	6.56
Pal	PalPal	PP	P	P	P	14	9	9	18	4.24
Pal	PalPal	PP	P	P	P	14	14	14	28	5.29
Pal	PalPal	PP	P	P	P	14	8	12	20	4.47
Pal	PalPal	PP	P	P	P	14	15	20	35	5.92
Pal	PalPal	PP	P	P	P	14	1	9	10	3.16
Pal	PalPal	PP	P	P	P	14	17	14	31	5.57
Pal	PalPal	PP	P	P	P	14	19	17	36	6.00
Pal	PalPal	PP	P	P	P	14	19	18	37	6.08
Pal	PalPal	PP	P	P	P	14	8	6	14	3.74
Pal	PalPal	PP	P	P	P	14	22	10	32	5.66
Pal	PalPal	PP	P	P	P	14	2	3	5	2.24
Pal	PalPal	PP	P	P	P	14	16	22	38	6.16
Pal	PalPal	PP	P	P	P	14	19	24	43	6.56
Pal	PalPal	PP	P	P	P	14	9	9	18	4.24
Pal	PalPal	PP	P	P	P	14	14	14	28	5.29
Pal	PalPal	PP	P	P	P	14	8	12	20	4.47
Pal	PalPal	PP	P	P	P	14	15	20	35	5.92
Pal	PalPal	PP	P	P	P	14	1	9	10	3.16

Appendix B. R Statistics Scripts

R Script for a Linear Mixed Model of *D. ananassae* compared to *D. pallidosa* with date as a random effect examining the total number of offspring produced by parental crosses. The output will produce an ANOVA between the full and reduced models, the mean of the data, the variance of the data, and the standard deviation of the data. The data set presented in Appendix A was manipulated to examine just the parental crosses between D. ananassae and D. pallidosa. To do this, every cross was re-categorized according to the new relationships (removing hybrid crosses from Pop).

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(SqRtTOS~Pop +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata)
anova(model1full,model1reduced,test="Chisq")
with(model1full, tapply(SqRtTOS, Pop,mean))
with(model1full, tapply(SqRtTOS, Pop,var))
with(model1full, tapply(SqRtTOS, Pop,sd))
qqnorm(resid(model1full))
qqline(resid(model1full))
```

R Script for a Linear Mixed Model of *D. ananassae* and *Drosophila* from Apia, Samoa lumped together compared to *D. pallidosa* with date as a random effect examining the total number of offspring produced. The output will produce an ANOVA between the full and reduced models, the mean of the data, the variance of the data, and the standard deviation of the data.

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
```

```
model1full <-lmer(TOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(TOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model2reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model3full <-lmer(TOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
model3reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(TOS, Pop1,mean))
with(model1full, tapply(TOS, Pop1,var))
with(model1full, tapply(TOS, Pop1,sd))
with(model2full, tapply(TOS, Pop2,mean))
with(model2full, tapply(TOS, Pop2,var))
with(model1full, tapply(TOS, Pop2,sd))
with(model3full, tapply(TOS, Pop3,mean))
with(model3full, tapply(TOS, Pop3,var))
with(model1full, tapply(TOS, Pop3,sd))
```

R Script for a Linear Mixed Model of D. ananassae and Drosophila from Apia, Samoa lumped together compared to D. pallidosa with date as a random effect examining the square root of the total number of offspring produced. The output will produce an ANOVA between the full and reduced models, the mean of the data, the variance of the data, the standard deviation of the data, and whisker plots of the normality curve.

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(SqRtTOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(SqRtTOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model2reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model3full <-lmer(SqRtTOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
```

```
model3reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(SqRtTOS, Pop1,mean))
with(model1full, tapply(SqRtTOS, Pop1,var))
with(model1full, tapply(SqRtTOS, Pop1,sd))
with(model2full, tapply(SqRtTOS, Pop2,mean))
with(model2full, tapply(SqRtTOS, Pop2,var))
with(model1full, tapply(SqRtTOS, Pop2,sd))
with(model3full, tapply(SqRtTOS, Pop3,mean))
with(model3full, tapply(SqRtTOS, Pop3,var))
with(model1full, tapply(SqRtTOS, Pop3,sd))
qqnorm(resid(model1full))
qqline(resid(model1full))
qqnorm(resid(model2full))
qqline(resid(model2full))
qqnorm(resid(model3full))
qqline(resid(model3full))
```

The data set presented in Appendix A was manipulated to examine the lumping of *D. pallidosa* with *Drosophila* collected from Apia, Samoa compared to the remaining *D. ananassae* populations in order to determine if the Apia, Samoan population of *Drosophila* were more related to *D. pallidosa* than to *D. ananassae*. To do this, every cross was re-categorized according to the new relationships; the Apia populations in in Appendix A were recorded as *D. pallidosa*, creating new combinations of hybrid from the first data set.

R Script for a Linear Mixed Model of *D. pallidosa* and *Drosophila* from Apia, Samoa lumped together compared to *D. ananassae* with date as a random effect examining the total number of offspring produced.

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(TOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(TOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model2reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model3full <-lmer(TOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
model3reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(TOS, Pop1,mean))
with(model1full, tapply(TOS, Pop1,var))
with(model1full, tapply(TOS, Pop1,sd))
with(model2full, tapply(TOS, Pop2,mean))
with(model2full, tapply(TOS, Pop2,var))
with(model1full, tapply(TOS, Pop2,sd))
with(model3full, tapply(TOS, Pop3,mean))
with(model3full, tapply(TOS, Pop3,var))
with(model1full, tapply(TOS, Pop3,sd))
```

R Script for a Linear Mixed Model of *D. ananassae* and *Drosophila* from Apia; Samoa lumped together compared to *D. pallidosa* with date as a random effect examining the square root of the total number of offspring produced.

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(SqRtTOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(SqRtTOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model2reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model3full <-lmer(SqRtTOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
model3reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(SqRtTOS, Pop1,mean))
with(model1full, tapply(SqRtTOS, Pop1,var))
with(model1full, tapply(SqRtTOS, Pop1,sd))
with(model2full, tapply(SqRtTOS, Pop2,mean))
with(model2full, tapply(SqRtTOS, Pop2,var))
with(model1full, tapply(SqRtTOS, Pop2,sd))
with(model3full, tapply(SqRtTOS, Pop3,mean))
with(model3full, tapply(SqRtTOS, Pop3,var))
with(model1full, tapply(SqRtTOS, Pop3,sd))
qqnorm(resid(model1full))
qqline(resid(model1full))
qqnorm(resid(model2full))
qqline(resid(model2full))
qqnorm(resid(model3full))
qqline(resid(model3full))
```

The data set presented in Appendix A was manipulated to examine whether the Apia, Somoan population of *Drosophila* had an effect on the overall viability data presented between *D. pallidosa* and *D. ananassae*. To accomplish this, all of the data

referencing the Apia, Samoan population was removed from the data set. This left only *D. pallidosa* compared to *D. ananassae*.

R Script for a Linear Mixed Model of *D. pallidosa* compared to *D. ananassae* with date as a random effect examining the total number of offspring produced.

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(TOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(TOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model2reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model3full <-lmer(TOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
model3reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(TOS, Pop1,mean))
with(model1full, tapply(TOS, Pop1,var))
with(model1full, tapply(TOS, Pop1,sd))
with(model2full, tapply(TOS, Pop2,mean))
with(model2full, tapply(TOS, Pop2,var))
with(model1full, tapply(TOS, Pop2,sd))
with(model3full, tapply(TOS, Pop3,mean))
with(model3full, tapply(TOS, Pop3,var))
with(model1full, tapply(TOS, Pop3,sd))
```

R Script for a Linear Mixed Model of *D. pallidosa* compared to *D. ananassae* with date as a random effect examining the total number of offspring produced.

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(SqRtTOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
```

```
model1reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(SqRtTOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model2reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model3full <-lmer(SqRtTOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
model3reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(SqRtTOS, Pop1,mean))
with(model1full, tapply(SqRtTOS, Pop1,var))
with(model1full, tapply(SqRtTOS, Pop1,sd))
with(model2full, tapply(SqRtTOS, Pop2,mean))
with(model2full, tapply(SqRtTOS, Pop2,var))
with(model1full, tapply(SqRtTOS, Pop2,sd))
with(model3full, tapply(SqRtTOS, Pop3,mean))
with(model3full, tapply(SqRtTOS, Pop3,var))
with(model1full, tapply(SqRtTOS, Pop3,sd))
qqnorm(resid(model1full))
qqline(resid(model1full))
qqnorm(resid(model2full))
qqline(resid(model2full))
qqnorm(resid(model3full))
qqline(resid(model3full))
```

The data set presented in Appendix A was manipulated to examine the lumping of *D. pallidosa* with *D. ananassae* compared to *Drosophila* collected from Apia, Samoa in order to determine if the Apia, Samoan population of *Drosophila* were more related to *D. pallidosa* than to *D. ananassae*. To do this, every cross was re-categorized according to the new relationships; the Apia populations in in Appendix A were recorded as local (L) while *D. ananassae* and *D. pallidosa* were recorded as species (S), creating new combinations of hybrid from the first data set.

R Script for a Linear Mixed Model of *D. ananassae* and *D. pallidosa* lumped together compared to *Drosophila* collected from Apia, Samoa with date as a random effect examining the total number of offspring produced.

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(TOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(TOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HL"|Pop2=="HS"))
model2reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HL"|Pop2=="HS"))
model3full <-lmer(TOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
model3reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(TOS, Pop1,mean))
with(model1full, tapply(TOS, Pop1,var))
with(model2full, tapply(TOS, Pop2,mean))
with(model2full, tapply(TOS, Pop2,var))
with(model3full, tapply(TOS, Pop3,mean))
with(model3full, tapply(TOS, Pop3,var))
```

R Script for a Linear Mixed Model of *D. ananassae* and *D. pallidosa* lumped together compared to *Drosophila* collected from Apia, Samoa with date as a random effect examining the square root of total number of offspring produced

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(SqRtTOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(SqRtTOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HL"|Pop2=="HS"))
model2reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HL"|Pop2=="HS"))
model3full <-lmer(SqRtTOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
```

```
subset=(Pop3=="hm"|Pop3=="hf"))
model3reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(SqRtTOS, Pop1,mean))
with(model1full, tapply(SqRtTOS, Pop1,var))
with(model1full, tapply(SqRtTOS, Pop1,sd))
with(model2full, tapply(SqRtTOS, Pop2,mean))
with(model2full, tapply(SqRtTOS, Pop2,var))
with(model2full, tapply(SqRtTOS, Pop2,sd))
with(model3full, tapply(SqRtTOS, Pop3,mean))
with(model3full, tapply(SqRtTOS, Pop3,var))
with(model3full, tapply(SqRtTOS, Pop3,sd))
qqnorm(resid(model1full))
qqline(resid(model1full))
qqnorm(resid(model2full))
qqline(resid(model2full))
qqnorm(resid(model3full))
qqline(resid(model3full))
```

The data set presented in Appendix A was manipulated to examine whether there was any postzygotic reproductive isolation between the proposed ancestral population (Bogor, Java) and the other, peripheral populations of *D. ananassae* and *D. pallidosa*. To accomplish this, every cross was re-categorized according to the new relationships; the Bogor populations in in Appendix A were recorded as local (B) while *D. ananassae* and *D. pallidosa* from the remaining populations were recorded as peripheral (E), creating new combinations of hybrids from the first data set.

R Script for a Linear Mixed Model of ancestral populations compared to peripheral populations with date as a random effect examining the total number of offspring produced.

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(TOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(TOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HB"|Pop2=="HE"))
model2reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HB"|Pop2=="HE"))
model3full <-lmer(TOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
model3reduced <- lmer(TOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(TOS, Pop1,mean))
with(model1full, tapply(TOS, Pop1,var))
with(model2full, tapply(TOS, Pop2,mean))
with(model2full, tapply(TOS, Pop2,var))
with(model3full, tapply(TOS, Pop3,mean))
with(model3full, tapply(TOS, Pop3,var))
```

R Script for a Linear Mixed Model of ancestral populations compared to peripheral populations with date as a random effect examining the square root of the total number of offspring produced.

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(SqRtTOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(SqRtTOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HB"|Pop2=="HE"))
model2reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HB"|Pop2=="HE"))
```

```
model3full <-lmer(SqRtTOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
model3reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(SqRtTOS, Pop1,mean))
with(model1full, tapply(SqRtTOS, Pop1,var))
with(model2full, tapply(SqRtTOS, Pop2,mean))
with(model2full, tapply(SqRtTOS, Pop2,var))
with(model3full, tapply(SqRtTOS, Pop3,mean))
with(model3full, tapply(SqRtTOS, Pop3,var))
qqnorm(resid(model1full))
qqline(resid(model1full))
qqnorm(resid(model2full))
qqline(resid(model2full))
qqnorm(resid(model3full))
```

The data set presented in Appendix A was manipulated to examine whether there was any postzygotic reproductive isolation between the Apia, Samoa population of *Drosophila* and *D. ananassae* collected from Bogor, Java. To accomplish this, every cross was re-categorized according to the new relationships; the Bogor populations in in Appendix A were recorded as B while the Apia, Samoa population was recorded as A, creating new combinations of hybrids from the first data set.

R Script for a Linear Mixed Model of Apia, Samoan populations of *Drosophila* compared to Bogor, Java populations with date as a random effect examining the total number of offspring produced.

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(SqRtTOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(SqRtTOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
```

```
subset=(Pop2=="HA"|Pop2=="HB"))
model2reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HB"))
model3full <-lmer(SqRtTOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
model3reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(SqRtTOS, Pop1,mean))
with(model1full, tapply(SqRtTOS, Pop1,var))
with(model1full, tapply(SqRtTOS, Pop1,sd))
with(model2full, tapply(SqRtTOS, Pop2,mean))
with(model2full, tapply(SqRtTOS, Pop2,var))
with(model1full, tapply(SqRtTOS, Pop3,sd))
with(model3full, tapply(SqRtTOS, Pop3,mean))
with(model3full, tapply(SqRtTOS, Pop3,var))
with(model1full, tapply(SqRtTOS, Pop3,sd))
qqnorm(resid(model1full))
qqline(resid(model1full))
qqnorm(resid(model2full))
qqline(resid(model2full))
qqnorm(resid(model3full))
qqline(resid(model3full))
```

The data set presented in Appendix A was manipulated to examine whether there was any postzygotic reproductive isolation between the Apia, Samoa population of *Drosophila* and *D. ananassae* collected from Nadi, Fiji. To accomplish this, every cross was re-categorized according to the new relationships; the Nadi populations in in Appendix A were recorded as N while the Apia, Samoa population was recorded as A, creating new combinations of hybrids from the first data set.

R Script for a Linear Mixed Model of Apia, Samoan populations of *Drosophila* compared to Nadi, Fiji populations with date as a random effect examining the total number of offspring produced.

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(SqRtTOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(SqRtTOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HN"))
model2reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HN"))
model3full <-lmer(SqRtTOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
model3reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(SqRtTOS, Pop1,mean))
with(model1full, tapply(SqRtTOS, Pop1,var))
with(model1full, tapply(SqRtTOS, Pop1,sd))
with(model2full, tapply(SqRtTOS, Pop2,mean))
with(model2full, tapply(SqRtTOS, Pop2,var))
with(model1full, tapply(SqRtTOS, Pop3,sd))
with(model3full, tapply(SqRtTOS, Pop3,mean))
with(model3full, tapply(SqRtTOS, Pop3,var))
with(model1full, tapply(SqRtTOS, Pop3,sd))
qqnorm(resid(model1full))
qqline(resid(model1full))
qqnorm(resid(model2full))
qqline(resid(model2full))
qqnorm(resid(model3full))
qqline(resid(model3full))
```

The data set presented in Appendix A was manipulated to examine whether there was any postzygotic reproductive isolation between the Apia, Samoa population of *Drosophila* and *D. pallidosa* collected from Nadi, Fiji. To accomplish this, every cross was re-categorized according to the new relationships; the Nadi populations in in

Appendix A were recorded as P while the Apia, Samoa population was recorded as A, creating new combinations of hybrids from the first data set.

R Script for a Linear Mixed Model of Apia, Samoan populations of *Drosophila* compared to Nadi, Fiji populations of *D. pallidosa* with date as a random effect examining the total number of offspring produced.

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(SqRtTOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(SqRtTOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model2reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HP"))
model3full <-lmer(SqRtTOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
model3reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(SqRtTOS, Pop1,mean))
with(model1full, tapply(SqRtTOS, Pop1,var))
with(model1full, tapply(SqRtTOS, Pop1,sd))
with(model2full, tapply(SqRtTOS, Pop2,mean))
with(model2full, tapply(SqRtTOS, Pop2,var))
with(model1full, tapply(SqRtTOS, Pop3,sd))
with(model3full, tapply(SqRtTOS, Pop3,mean))
with(model3full, tapply(SqRtTOS, Pop3,var))
with(model1full, tapply(SqRtTOS, Pop3,sd))
qqnorm(resid(model1full))
qqline(resid(model1full))
qqnorm(resid(model2full))
qqline(resid(model2full))
qqnorm(resid(model3full))
qqline(resid(model3full))
```

The data set presented in Appendix A was manipulated to examine whether there was any postzygotic reproductive isolation between the Apia, Samoa population of *Drosophila* and *D. ananassae* collected from Trinity Beach, Australia. To accomplish this, every cross was re-categorized according to the new relationships; the Trinity Beach population in in Appendix A was recorded as T while the Apia, Samoa population was recorded as A, creating new combinations of hybrids from the first data set.

R Script for a Linear Mixed Model of Apia, Samoan populations of *Drosophila* compared to Trinity Beach, Australia populations of *D. ananassae* with date as a random effect examining the total number of offspring produced.

```
flydata <- read.table( file.choose(), header=TRUE,sep="\t")
data=flydata
model1full <-lmer(SqRtTOS~Pop1 +(1|Date), REML=FALSE, data=flydata)
model1reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata)
model2full <-lmer(SqRtTOS~Pop2 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HT"))
model2reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop2=="HA"|Pop2=="HT"))
model3full <-lmer(SqRtTOS~Pop3 +(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
model3reduced <- lmer(SqRtTOS~1+(1|Date), REML=FALSE, data=flydata,
subset=(Pop3=="hm"|Pop3=="hf"))
anova(model1full,model1reduced,test="Chisq")
anova(model2full,model2reduced,test="Chisq")
anova(model3full,model3reduced,test="Chisq")
with(model1full, tapply(SqRtTOS, Pop1,mean))
with(model1full, tapply(SqRtTOS, Pop1,var))
with(model1full, tapply(SqRtTOS, Pop1,sd))
with(model2full, tapply(SqRtTOS, Pop2,mean))
with(model2full, tapply(SqRtTOS, Pop2,var))
with(model1full, tapply(SqRtTOS, Pop3,sd))
with(model3full, tapply(SqRtTOS, Pop3,mean))
with(model3full, tapply(SqRtTOS, Pop3,var))
```

```
with(model1full, tapply(SqRtTOS, Pop3,sd))
qqnorm(resid(model1full))
qqline(resid(model1full))
qqnorm(resid(model2full))
qqline(resid(model2full))
qqnorm(resid(model3full))
qqline(resid(model3full))
```

Appendix C. ANOVA Results from R Statistics

Table 1C: ANOVA of Square Root Transformed Data of *D. ananassae* compared to *D. pallidosa* parental crosses

Anova	Df	AIC	BIC	logLik	Chisq	Chi Df	Pr(>Chisq)	Significance
model1reduced (SqRtTOS)	3	3441.0	3455.1	-1717.5				
model1full	4	3440.0	3458.7	-1716.0	3.0231	1	0.08208	

* = P < 0.05; ** = P < .005; *** P < 0.0005

Table 2C: ANOVA of Square Root Transformed Data of Lumping *Drosophila* from Apia, Samoa with *D. ananassae* compared to *D. pallidosa*

Anova	Df	AIC	BIC	logLik	Chisq	Chi Df	Pr(>Chisq)	Significance
model1reduced (SqRtTOS)	3	4262.7	4277.4	-2128.4				
model1full	4	4254	4273.6	-2123	10.675	1	0.001086	**
model2reduced (SqRtTTOS)	3	809.33	819.18	-401.66				
model2full	4	811.31	824.45	-401.66	0.0131	1	0.9088	
model3reduced (SqRtTTOS)	3	873.54	883.61	-433.77				
model3full	4	873.03	886.45	-432.51	2.5148	1	0.1128	

* = P < 0.05; ** = P < .005; *** P < 0.0005

TABLE 3C: ANOVA of Square Root Transformed Data of Lumping *Drosophila* from Apia, Samoa with *D. pallidosa* compared to *D. ananassae*

Anova	Df	AIC	BIC	logLik	Chisq	Chi Df	Pr(>Chisq)	Significance
model1reduced (SqRtTOS)	3	4262.7	4277.4	-2128.4				
model1full	4	4182	4201.6	-2087	82.722	1	< 2.2e-16	***
model2reduced (SqRtTTOS)	3	1721.56	1733.68	-857.78				
model2full	4	1723.56	1739.72	-857.78	5.00E-04	1	0.9822	
model3reduced (SqRtTTOS)	3	1721.56	1733.68	-857.78				
model3full	4	1719.91	1736.07	-855.95	3.6513	1	0.05603	

* = P < 0.05; ** = P < .005; *** P < 0.0005

TABLE 4C: ANOVA of Square Root Transformed Data of *D. pallidosa* compared to *D. ananassae* (without *Drosophila* from Apia, Samoa).

Anova	Df	AIC	BIC	logLik	Chisq	Chi Df	Pr(>Chisq)	Significance
model1reduced (SqRtTOS)	3	2941.3	2955	-1467.7				
model1full	4	2917.5	2935.7	-1454.7	25.855	1	3.68E-07	***
model2reduced (SqRtTTOS)	3	692.77	702.14	-343.39				
model2full	4	694.61	707.11	-343.31	0.1621	1	0.6872	
model3reduced (SqRtTTOS)	3	678.48	687.78	-336.24				
model3full	4	678.74	691.13	-335.37	1.7479	1	0.1861	

* = P < 0.05; ** = P < .005; *** P < 0.0005

TABLE 5C: ANOVA of Square Root Transformed Data of Lumping *D. ananassae* and *D. pallidosa* compared to *Drosophila* from Apia, Samoa

Anova	Df	AIC	BIC	logLik	Chisq	Chi Df	Pr(>Chisq)	Significance
model1reduced (SqRtTOS)	3	4262.7	4277.4	-2128.4				
model1full	4	4161.8	4181.4	-2076.9	102.91	1	< 2.2e-16	***
model2reduced (SqRtTTOS)	3	1162.69	1173.65	-578.35				
model2full	4	1162.83	1177.44	-577.42	1.8626	1	0.1723	
model3reduced (SqRtTTOS)	3	1162.69	1173.65	-578.35				
model3full	4	1160.48	1175.09	-576.24	4.2121	1	0.04014	*

* = P < 0.05; ** = P < .005; *** P < 0.0005

TABLE 6C: ANOVA of Square Root Transformed Data of ancestral populations compared to peripheral populations

Anova	Df	AIC	BIC	logLik	Chisq	Chi Df	Pr(>Chisq)	Significance
model1reduced (SqRtTOS)	3	4262.7	4277.4	-2128.4				
model1full	4	4263.1	4282.7	-2127.5	1.6179	1	0.2034	
model2reduced (SqRtTTOS)	3	1257.09	1268.15	-625.54				
model2full	4	1257.11	1271.86	-624.55	1.9799	1	0.1594	
model3reduced (SqRtTTOS)	3	1257.09	1268.15	-625.54				
model3full	4	1251.29	1266.03	-621.64	7.8025	1	0.005217	**

* = P < 0.05; ** = P < .005; *** P < 0.0005

Appendix D. Descriptive Statistics

Legend: PA = Parental *D. ananassae*, PP = Parental *D. pallidosa*; H = hybrid; P = Parent; HA = Hybrid backcrossed to *D. ananassae*; HP = hybrid backcrossed to *D. pallidosa*; hf = female of cross is hybrid; hm = male of cross is hybrid; HL = Hybrid backcrossed to ancestral population; HE = hybrid backcrossed to peripheral population; HB = hybrid backcrossed to Bogor, Java; HN = Hybrid backcrossed to Nadi, Fiji; HT = hybrid backcrossed to Trinity Beach, Australia

TABLE 1D: Means, Variance, and Standard Deviation of Square Root Transformed Data of *D. ananassae* compared to *D. pallidosa* parental crosses

	Mean	Variance	TOS	sd
PA	6.437982	4.878683	40.49239	2.208774
PP	4.923700	3.486234	43.52625	1.867146

TABLE 2D: Means, Variance, and Standard Deviation of Square Root Transformed Data of Lumping *Drosophila* from Apia, Samoa with *D. ananassae* compared to *D. pallidosa*

	Mean	Variance	TOS	sd
H	6.044822	3.972676	40.49239	23.5681
P	6.210839	4.957921	43.52625	27.10485
HA	6.26115	5.870173	43.86364	26.75439
HP	4.715225	3.34238	37.77064	20.36411
hf	5.913778	5.790832	38.51818	20.89894
hm	3.577944	4.965936	38.45098	26.53099

TABLE 3D:
Means, Variance, and Standard Deviation of Square Root Transformed Data of Lumping *Drosophila* from Apia, Samoa with *D. pallidosa* compared to *D. ananassae*

	Mean	Variance	TOS	sd
H	5.744165	3.952074	36.9381	22.56597
P	6.493851	5.124737	47.28596	28.19623
HA	5.956482	4.466428	39.92523	25.09782
HP	5.523603	3.340984	33.83495	19.16582
hf	5.80849	3.687898	37.4093	20.23991
hm	5.676702	4.239639	36.4439	24.81309

TABLE 4D: Means, Variance, and Standard Deviation of Square Root Transformed Data of *D. pallidosa* compared to *D. ananassae* (without *Drosophila* from Apia, Samoa).

	Mean	Variance	TOS	sd
H	6.202404	3.952518	42.39881	23.77619
P	6.732056	5.049606	50.36075	28.35745
HB	6.378175	4.830107	45.44595	27.201
HE	6.064032	3.262223	40	20.52169
hf	6.090082	3.548246	40.6	44.43478
hm	6.310262	4.683251	20.84155	27.64506

TABLE 5D: Means, Variance, and Standard Deviation of Square Root Transformed Data of Lumping *D. ananassae* with *D. pallidosa* compared to *Drosophila* from Apia, Samoa

	Mean	Variance	TOS	sd
H	5.378767	3.657133	32.57544	20.7522
P	6.497967	4.85346	47.07022	27.36071
HL	5.105002	3.047006	29.08462	17.13921
HS	5.608377	4.07563	35.50323	23.00462
hf	5.494942	3.597911	33.76923	19.17385
hm	5.238277	3.721078	31.13178	22.50534

TABLE 6D: Means, Variance, and Standard Deviation of Square Root Transformed Data of ancestral populations compared to peripheral populations.

	Mean	Variance	TOS	sd
H	6.359273	4.575159	45	27.34138
P	6.101875	4.82956	42.05556	26.05127
HB	6.563767	4.245633	47.30247	26.62509
HE	6.110189	4.897907	42.19549	28.03446
hf	6.626694	4.58756	48.46715	26.10895
hm	6.127394	4.477045	41.99367	28.1032

Appendix E. Plot of Normality

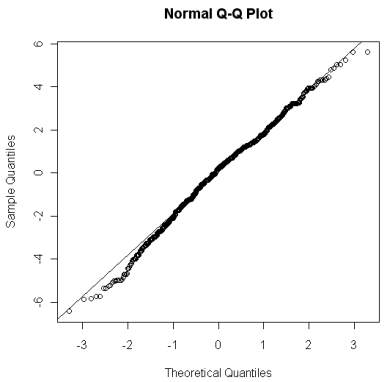

Figure 1E: Normality Q-Map of Square Root Transformed Data Model 2 of Square Root Transformed Data of Lumping *Drosophila* from Apia, Samoa with *D. ananassae* compared to *D. pallidosa*

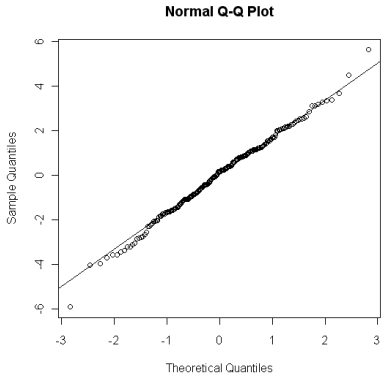

Figure 2E: Normality Q-Map of Square Root Transformed Data Model 2 of Square Root Transformed Data of Lumping *Drosophila* from Apia, Samoa with *D. ananassae* compared to *D. pallidosa*

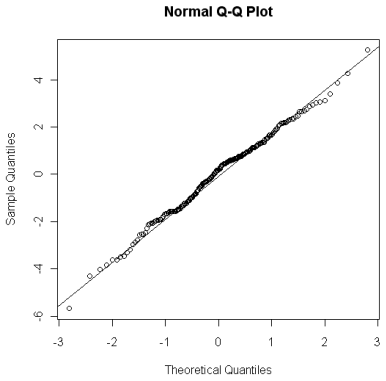

Figure 3E: Normality Q-Map of Square Root Transformed Data Model 3 of Square Root Transformed Data of Lumping *Drosophila* from Apia, Samoa with *D. ananassae* compared to *D. pallidosa*

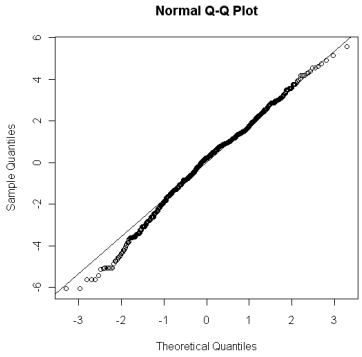

Figure 4E: Normality Q-Map of Square Root Transformed Data Model 1 of Square Root Transformed Data of Lumping *Drosophila* from Apia, Samoa with *D. pallidosa* compared to *D. ananassae*

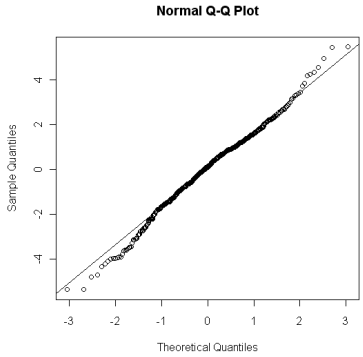

Figure 5E: Normality Q-Map of Square Root Transformed Data Model 2 of Square Root Transformed Data of Lumping *Drosophila* from Apia, Samoa with *D. pallidosa* compared to *D. ananassae*

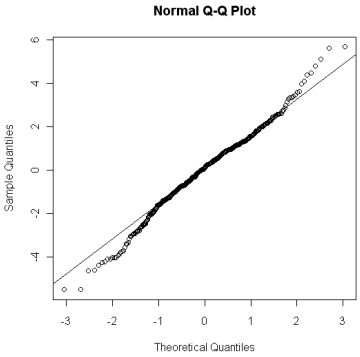

Figure 6E: Normality Q-Map of Square Root Transformed Data Model 3 of Square Root Transformed Data of Lumping *Drosophila* from Apia, Samoa with *D. pallidosa* compared to *D. ananassae*

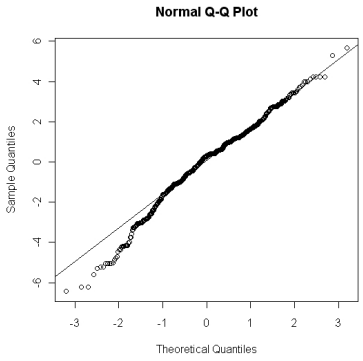

Figure 7E: Normality Q-Map of Square Root Transformed Data Model 1 of Square Root Transformed Data of *D. pallidosa* compared to *D. ananassae* (without *Drosophila* from Apia, Samoa).

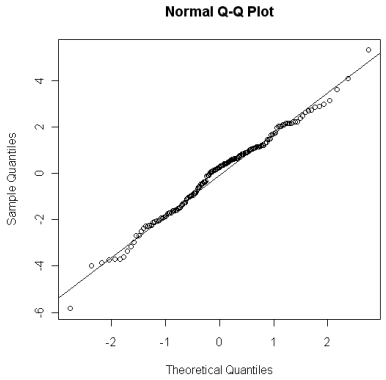

Figure 8E: Normality Q-Map of Square Root Transformed Data Model 2 of Square Root Transformed Data of *D. pallidosa* compared to *D. ananassae* (without *Drosophila* from Apia, Samoa).

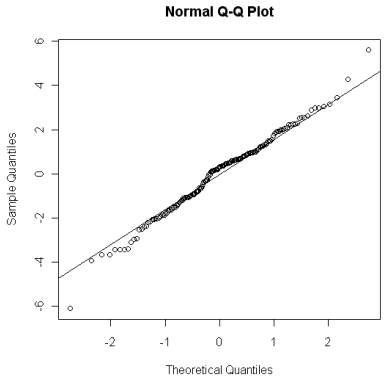

Figure 9E: Normality Q-Map of Square Root Transformed Data Model 3 of Square Root Transformed Data of *D. pallidosa* compared to *D. ananassae* (without *Drosophila* from Apia, Samoa).

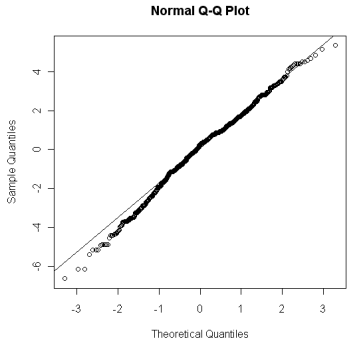

Figure 10E: Normality Q-Map of Square Root Transformed Data Model 1 of Square Root Transformed Data of Lumping *D. ananassae* with *D. pallidosa* compared to *Drosophila* from Apia, Samoa

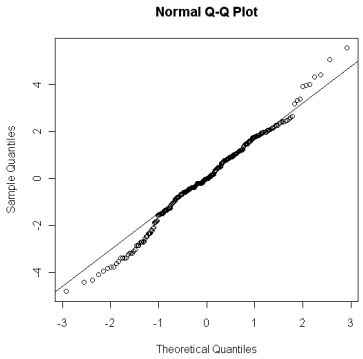

Figure 11E: Normality Q-Map of Square Root Transformed Data Model 2 of Square Root Transformed Data of Lumping *D. ananassae* with *D. pallidosa* compared to *Drosophila* from Apia, Samoa

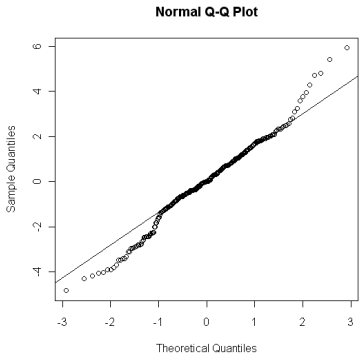

Figure 12E: Normality Q-Map of Square Root Transformed Data Model 3 of Square Root Transformed Data of Lumping *D. ananassae* with *D. pallidosa* compared to *Drosophila* from Apia, Samoa

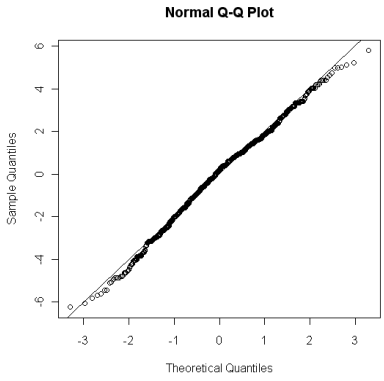

Figure 13E: Normality Q-Map of Square Root Transformed Data Model 1 of ancestral populations compared to peripheral populations

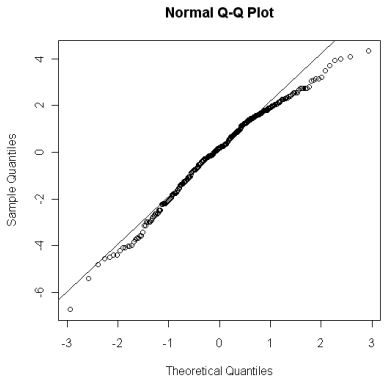

Figure 14E: Normality Q-Map of Square Root Transformed Data Model 2 of ancestral populations compared to peripheral populations

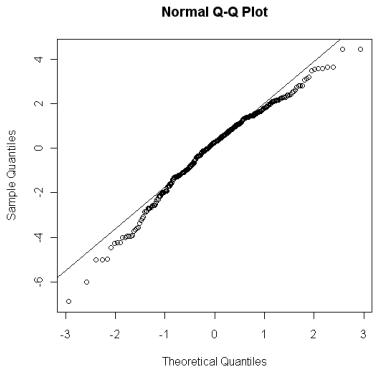

Figure 15E: Normality Q-Map of Square Root Transformed Data Model 3 of ancestral populations compared to peripheral populations

Appendix F: Samoan Cross Raw Data

Table 1F: Raw Data of Apia, Samoa lines crossed to Malololelei, Samoa lines
Key: M = line created from female collected from Malololelei, Samoa; A = line created from female collected from Apia, Samoa; Population = male crossed to female

Male Parent	Female Parent	Population	Experiment Date	# Male Offspring	# Female Offspring	Total # Offspring
M8	A32	MxA	4-Oct-05	13	11	24
M8	A32	MxA	4-Oct-05	11	8	19
M8	A32	MxA	4-Oct-05	18	12	30
M8	A32	MxA	4-Oct-05	9	11	20
M8	A32	MxA	4-Oct-05	22	12	34
M12	A77	MxA	4-Oct-05	20	11	31
M12	A77	MxA	4-Oct-05	24	27	51
M12	A77	MxA	4-Oct-05	12	9	21
M12	A77	MxA	4-Oct-05	16	9	25
M12	A77	MxA	4-Oct-05	13	13	26
M12	A77	MxA	4-Oct-05	11	6	17
M18	A56	MxA	4-Oct-05	10	9	19
M18	A56	MxA	4-Oct-05	13	9	22
M18	A56	MxA	4-Oct-05	6	9	15
M18	A56	MxA	4-Oct-05	11	10	21
M18	A56	MxA	4-Oct-05	22	14	36
M8	M12	MxM	4-Oct-05	9	13	22
M8	M12	MxM	4-Oct-05	12	14	26
M8	M12	MxM	4-Oct-05	11	12	23
M8	M12	MxM	4-Oct-05	21	17	38
M12	M18	MxM	4-Oct-05	10	8	18
M12	M18	MxM	4-Oct-05	11	6	17
M12	M18	MxM	4-Oct-05	17	12	29
M12	M18	MxM	4-Oct-05	15	17	32
M18	M8	MxM	4-Oct-05	19	12	31
M18	M8	MxM	4-Oct-05	15	14	29
M18	M8	MxM	4-Oct-05	14	11	25
M18	M8	MxM	4-Oct-05	13	10	23
A19	A32	AxA	4-Oct-05	11	11	22
A19	A32	AxA	4-Oct-05	16	9	25
A19	A32	AxA	4-Oct-05	27	13	40
A19	A32	AxA	4-Oct-05	13	9	22
A19	A32	AxA	4-Oct-05	9	10	19
A19	A32	AxA	4-Oct-05	13	9	22
A56	A77	AxA	4-Oct-05	20	15	35
A56	A77	AxA	4-Oct-05	25	22	47

A56	A77	AxA	4-Oct-05	14	14	28
A56	A77	AxA	4-Oct-05	12	13	25
A77	A56	AxA	4-Oct-05	14	12	26
A77	A56	AxA	4-Oct-05	17	21	38
A77	A56	AxA	4-Oct-05	19	11	30
A77	A56	AxA	4-Oct-05	8	9	17
A77	A56	AxA	4-Oct-05	23	13	36
A19	M12	AxM	4-Oct-05	22	11	33
A19	M12	AxM	4-Oct-05	11	14	25
A19	M12	AxM	4-Oct-05	11	6	17
A19	M12	AxM	4-Oct-05	17	13	30
A19	M12	AxM	4-Oct-05	9	10	19
A19	M12	AxM	4-Oct-05	21	15	36
A56	M77	AxM	4-Oct-05	19	11	30
A56	M77	AxM	4-Oct-05	13	10	23
A56	M77	AxM	4-Oct-05	12	9	21
A56	M77	AxM	4-Oct-05	14	13	27
A77	M18	AxM	4-Oct-05	9	8	17
A77	M18	AxM	4-Oct-05	16	9	25
A77	M18	AxM	4-Oct-05	5	8	13
A77	M18	AxM	4-Oct-05	11	8	19
A77	M18	AxM	4-Oct-05	21	14	35